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THE SIZE OF THE EXTRACELLULAR FLUID COMPARTMENT BEFORE AND AFTER MASSIVE INFUSIONS

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The idea seems to have been widely accepted that certain substances amongst which are chlorides and sulfocyanides can be used to estimate the size of the extracellular fluid compartment of the body as a whole or the size of the extracellular compartment of its various organs (1, 2, 3). The principle of the estimation involves two assumptions: 1, that such substances do not penetrate the cells, and 2, that in the fluids outside the cells they undergo and are maintained in uniform distribution. It can be easily shown that chlorides and sulfocyanides do enter the erythrocytes of the blood stream (1), but the adult red corpuscles are not true cells morphologically in that they contain no nuclei and there is reason for believing that their behavior is not necessarily that of true cells. On the other hand, experimental findings have shown with remarkable uniformity a constant fraction of the total body substance not invaded by chlorides and sulfocyanides (approximately 70 per cent by volume) and it has been argued that this can only represent that portion composed of cells.

The experiments now being reported have made use of the above assumptions in attacking the general problem of the physiologic response to massive infusions of approximately isotonic solutions of sodium chloride and glucose concerning which several reports from this laboratory have already been published (4, 5, 6). The present investigation is concerned with the estimation, by the chloride and sulfocyanide methods, of the normal size of the total extracellular fluid compartment of the body, the extracellular compartment of its component organs, and the manner and extent of modification of the size of these compartments by massive infusions.

METHODS AND CALCULATIONS. As in previous investigations, cats were used as experimental animals. They were anesthetized by injecting

intraperitoneally either dial[1] (60 mgm./kgm. body weight) or phenobarbital (40 mgm./kgm. body weight). A suitable accurately weighed quantity of sulfocyanide (usually about 140 mgm./kgm. body weight) was then given by vein and 30 minutes were allowed for uniform distribution throughout the sulfocyanide available volume of the animal. At the end of this time 10 ml. of blood were withdrawn from the carotid artery and set aside for subsequent sulfocyanide (7) and chloride (8) determinations. Control animals (not infused) were immediately sacrificed by asphyxia and their tissues taken for analysis. The control group comprises 14 animals.

In the case of the NaCl solution the amount given was such as to give a total infused volume of 400–550 ml./kgm. initial body weight and a retained volume of 375 ml./kgm. initial body weight. Glucose solutions were given in such amounts as to give a total infused volume of 300–475 ml./kgm. initial body weight and a retained volume of 275 ml./kgm.

TABLE 1

Volume of extracellular water in the cat on the basis of sulfocyanide distribution and total chloride content

EXPERIMENT	WEIGHT	AVAILABLE WATER IN ML./KGM. CAT	
		Sulfocyanide	Chloride
	kgm.		
A	1.98	288	316
B	1.65	288	320

initial body weight. These amounts represent volumes certainly not lethal but definitely approaching lethal values.

Thirty minutes were allowed after the termination of the infusion for uniform distribution of the infusate. Ten milliliters of blood were then withdrawn from the carotid artery and were set aside for subsequent chloride and sulfocyanide analysis. The animals were killed by asphyxia. Various organs were removed promptly, blotted to remove excess blood and accurately weighed. These were then dried to constant weight in an oven maintained at 105°C. and their dry weight determined. The fat was removed by extraction with ether and the fat free weight determined. The dried, fat free tissue was subsequently dissolved in normal KOH solution from which aliquot portions were taken for chloride (9) and sulfocyanide (10) analysis.

In all cases excreta (feces, urine and vomitus) were collected and analyzed for chlorides and sulfocyanide.

[1] Supplied by courtesy of Ciba Pharmaceutical Products, Inc. Lafayette Park, Summit, N. J.

In two additional experiments after determining the sulfocyanide available volume of the animals, the whole cat was dissolved in normal KOH solution. The chloride content of the dissolved animal was determined and the chloride available volume calculated. These volumes were then compared (table 1).

The following calculations have been made and tabulated:

Sulfocyanide available water of the uninfused cat in ml./kgm. body weight (AW_S):

$$(1)\qquad \frac{\text{mM sulfocyanide injected/kgm. body weight} - \text{mM sulfocyanide excreted/kgm. body weight}}{\text{mM sulfocyanide/ml. serum}}$$

Sulfocyanide available water of the infused cat in ml./kgm. final [2] body weight (AW'_S);

$$(2)\qquad \frac{\text{mM sulfocyanide injected/kgm. final body weight} - \text{mM sulfocyanide excreted (and in peritoneal exudate)/kgm. final body weight}}{\text{mM sulfocyanide/ml. serum after infusion}}$$

Chloride available water of the infused cat in ml./kgm. final body weight (AW'_{Cl});

$$(3)\qquad \frac{\left\{\begin{matrix}\text{total sulfocyanide available water in ml./kgm. original body weight}\end{matrix}\right\} \times \left\{\begin{matrix}\text{serum chloride of the uninfused cat in mM/ml.}\end{matrix}\right\} + \left\{\begin{matrix}\text{[3] infused chloride retained in mM/kgm. original body weight}\end{matrix}\right\}}{\text{serum chloride of the infused cat in mM/ml.}}$$

Sulfocyanide available water of individual organs in ml./kgm. final body weight;

$$(4)\qquad \frac{\text{sulfocyanide in mM/kgm. organ}}{\text{sulfocyanide in mM/ml. serum}}$$

Chloride available water of individual organs in ml./kgm. final weight;

$$(5)\qquad \frac{\text{chloride in mM/kgm. organ}}{\text{chloride in mM/ml. serum}}$$

Calculated sulfocyanide available water of individual organs of infused cats in per cent total water following infusion:

$$(6)\qquad \frac{\left\{\begin{matrix}\text{sulfocyanide available water in ml./kgm. original weight} + \text{total increase in water content in ml./kgm. original weight}\end{matrix}\right\} \times 100}{\left\{\begin{matrix}1000 + \text{increase in water content in ml./kgm. original weight}\end{matrix}\right\} \times \text{water content in ml./gm. infused organ}}$$

Calculated chloride available water of individual organs of infused cats in per cent total water following infusion:

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[2] Final body weight, as used in this study, represents the original weight to which is added the volume of fluid infused less the volumes of fluid excreted by all excretory organs and that volume of fluid found free in the peritoneal cavity at the end of infusion.

[3] Chloride lost by excretion or passing into water in the peritoneal cavity during glucose infusion must be subtracted.

$$(7)\qquad \frac{\left\{\begin{array}{c}\text{chloride available water in}\\ \text{ml./kgm. original weight}\end{array} + \begin{array}{c}\text{total increase in water con-}\\ \text{tent in ml./kgm. original}\\ \text{weight}\end{array}\right\} \times 100}{\left\{\begin{array}{c}1000 + \text{increase in water content}\\ \text{in ml./kgm. original weight}\end{array}\right\} \times \begin{array}{c}\text{water content in ml./gm.}\\ \text{infused organ}\end{array}}$$

I. *The size of the chloride and sulfocyanide available volumes in the normal cat.* The total chloride available water of the cat is slightly greater than the sulfocyanide available water (table 1). This becomes especially

TABLE 2

Sulfocyanide and chloride available volumes of the organs in normal cats

ORGAN	NORMAL CHLORIDE VALUES					NORMAL SULFOCYANIDE VALUES				
	Experiment 3 Chloride available water			Average chloride available water (12 experiments)		Experiment 3 Sulfocyanide available water			Average sulfocyanide available water (12 experiments)	
	Chloride (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Sulfocyanide (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total
Blood serum†	127.0					5.70				
Gastrocnemius	19.7	155	20.8	124	16.2	0.96	169	22.6	162	21.2
Biceps femoris	20.4	161	21.2	132*	17.2	1.03	180	24.2	137*	17.9
Triceps brachii	19.0	150	19.5	133*	17.5	1.00	175	23.1	134*	17.7
Temporalis	20.6	162	22.0	153*	20.6	1.01	177	24.4	168*	22.6
Spinotrapezius	27.2	215	28.2	200*	26.0	1.33	233	30.6	202*	26.3
Sternomastoideus	41.3	325	44.5	248*	32.4	1.80	317	43.5	240*	31.7
Diaphragm	53.4	423	56.5	293*	38.0	2.70	475	63.5	329*	42.9
Lateral abdominal wall muscle	44.7	353	45.6	351*	45.7	1.83	322	41.7	359*	46.9
Rectus abdominis	60.0	473	60.0	375*	49.0	2.58	455	57.9	344*	45.0
Cardiac muscle	41.0	322	42.0	302	38.2	1.94	342	44.3	329	41.5
Liver	28.7	227	31.4	285	40.0					
Brain (cerebrum)	34.7	274	31.4	325	47.8	0.46	81	9.3	87	10.1
Duodenum	50.7	400	51.5	385	48.6	1.48	260	33.7	326	41.1
Colon	57.0	451	58.5	416	53.2	2.96	519	67.5	447	57.1
Spleen	52.6	414	54.0	426	54.4	2.38	420	54.5	374	47.8
Pancreas	81.5	642	84.5	407	52.0	1.92	336	44.2	327	41.8
Stomach	79.2	623	76.6	548	68.2	2.88	507	62.0	467	58.2
Lung	77.0	607	77.0	576	72.5	2.17	382	48.5	401	50.3
Kidney	97.5	767	98.5	586	75.0	2.96	519	66.0	447	57.2
Skin	99.5	785	115.5	684	102.0	4.52	793	117.0	602	90.0

* Average determinations from four experiments.

† Average chloride 127.0 mM/kgm. serum. Average sulfocyanide 5.96 mM/kgm. serum.

apparent when the various organs are separately analyzed and their chloride and sulfocyanide available water volumes are compared (table 2). The volume of water available for the distribution of chloride and sulfocyanide in a given organ varies somewhat from animal to animal. The distribution in the skin indicates a volume available to both of these substances equal to the total water content (total water content 67.03 ml./100 grams; calculated NaCNS available volume 60.2 ml./100 grams; calculated chloride available volume 68.4 ml./100 grams). The brain

has a chloride available water volume much in excess of that available to sulfocyanide. This apparently results from the slow penetration of sulfocyanide into the cerebrospinal tissue spaces, the water of which contains a slightly higher chloride concentration than that of the blood plasma. The general inertia of the cerebrospinal fluid to changes in the composition of the plasma following infusion is quite clearly demonstrable

TABLE 3a

Volume of chloride and sulfocyanide available water following infusion with 5 per cent glucose solution

ORGAN	EXPERIMENT 15 CHLORIDE AVAILABLE WATER			AVERAGE CHLORIDE AVAILABLE WATER (5 EXPERIMENTS)			EXPERIMENT 15 SULFOCYANIDE AVAILABLE WATER			AVERAGE SULFOCYANIDE AVAILABLE WATER (5 EXPERIMENTS)		
	Chloride (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water*	Sulfocyanide (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water†
Blood serum‡	60.2						3.50					
Gastrocnemius	10.0	167	21.8	164	21.2	19.3	1.05	300	39.0	339	44.0	24.1
Biceps femoris	11.2	186	24.0	169	21.9	20.7	1.01	289	37.6	257	33.2	21.4
Triceps brachii	13.2	220	28.8	174	22.5	23.4	0.89	255	33.4	336	43.6	23.6
Temporalis	20.0	332	42.5	240	31.0	32.7	1.53	438	56.0	412	52.0	34.4
Spinotrapezius	18.6	309	40.0	288	37.0	29.8	1.23	351	44.5	443	57.1	30.0
Sternomastoideus	31.7	526	66.5	458	57.0	48.3	2.72	777	97.2	543	67.7	47.5
Diaphragm	21.1	350	43.0	358	44.5	49.5	1.37	392	48.0	485	60.5	52.9
Lateral abdominal wall muscle	16.6	276	35.0	285	36.0	52.1	1.32	377	48.0	497	62.8	53.3
Rectus abdominis	22.4	372	47.2	373	47.8	54.0	1.73	495	62.8	458	58.7	50.0
Cardiac muscle	22.6	374	46.7	365	44.8	45.8	1.57	449	55.6	462	56.9	48.8
Liver	20.4	336	41.5	364	46.5	58.4						
Brain	35.7	592	69.0	573	67.0	35.6	0.64	183	21.3	220	25.7	6.8
Duodenum	39.4	654	81.1	565	70.1	52.7	2.70	770	94.5	655	81.5	46.0
Colon	35.4	588	68.4	650	78.0	66.4	2.86	819	95.0	819	93.5	69.2
Spleen	37.2	618	78.7	590	76.9	43.3	1.87	535	68.4	563	73.3	37.0
Pancreas	35.2	585	64.2	825	91.0	83.0	1.79	511	56.4	634	69.7	79.5
Stomach	39.2	651	74.3	755	88.0	78.0	2.50	715	81.8	563	65.8	71.9
Lung	34.9	580	65.4	550	62.7	85.8	2.58	738	83.0	768	87.5	73.6
Kidney	26.2	435	51.4	497	58.3	84.5	1.44	412	48.7	483	56.6	73.6
Skin	46.2	767	110.0	763	101.0	102.2	4.22	1200	173.0	980	130.0	94.0

* Obtained from equation 7.

† Obtained from equation 6.

‡ Average chloride 60.8 mM/kgm. serum. Average sulfocyanide 4.93 mM/kgm. serum.

and will be discussed presently. After the brain, the organs showing the greatest difference between chloride and sulfocyanide available water are lung, kidney and stomach. The kidney, under normal conditions, excretes very small quantities of sulfocyanide during the first few hours following injection. The concentration of this substance in the tubules must be relatively less than the concentration of chloride and would thus give a much lower volume of distribution than would be indicated by the

chloride content of the organ. The difference between the sulfocyanide and chloride available water in the stomach would indicate that about 14 per cent of the total chloride of this organ is in some of the gastric cells. Manery and Hastings (12) have recently reported the results of separate analyses of fundic and pyloric mucosa. They report similarly high chloride values for these tissues. Muscles differ a great deal from one

TABLE 3b

Volume of chloride and sulfocyanide available water following infusion with 1 per cent sodium chloride solution

ORGAN	EXPERIMENT 16 CHLORIDE AVAILABLE WATER			AVERAGE CHLORIDE AVAILABLE WATER (5 EXPERIMENTS)			EXPERIMENT 16 SULFOCYANIDE AVAILABLE WATER			AVERAGE SULFOCYANIDE AVAILABLE WATER (5 EXPERIMENTS)		
	Chloride (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water*	Sulfocyanide (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water†
Blood serum‡	136.5						2.99					
Gastrocnemius	25.0	183	23.8	241	30.7	27.3	0.57	191	25.0	229	29.2	31.7
Biceps femoris	25.4	186	23.8	217	27.4	28.4	0.62	207	25.7	215	27.2	28.9
Triceps brachii	30.7	225	29.0	254	32.3	29.6	0.67	224	28.9	250	31.8	29.7
Temporalis	43.6	320	41.6	345	43.6	39.4	0.92	307	40.2	329	41.5	40.9
Spinotrapezius	41.2	302	38.5	363	45.8	36.0	0.77	257	32.6	340	43.0	36.2
Sternomastoideus	64.5	472	58.5	462	56.8	52.0	0.82	274	33.5	412	50.7	51.3
Diaphragm	52.5	384	48.0	437	53.4	53.7	1.23	411	51.5	382	46.8	57.2
Lat. abd. wall muscle	53.0	388	48.8	392	49.0	54.2	1.17	392	49.0	410	51.5	54.8
Rectus abdominis	61.4	450	56.0	470	59.5	56.6	1.22	409	51.0	462	58.5	53.0
Cardiac muscle	54.9	402	50.5	435	53.5	45.7	0.99	331	41.3	368	45.3	48.9
Liver	38.6	283	37.4	332	43.7	52.6						
Brain	40.9	300	34.6	341	39.5	39.2	0.66	220	25.6	172	20.0	12.3
Duodenum	77.1	564	69.2	520	63.9	55.0	1.62	541	67.0	529	65.0	48.6
Colon	87.0	637	75.0	662	78.0	70.5	1.73	580	68.5	604	71.0	73.0
Spleen	52.4	384	49.0	459	59.3	50.3	1.32	440	56.9	464	60.0	43.2
Pancreas	106.0	776	82.5	884	94.0	86.7	1.47	492	61.2	469	50.0	84.5
Stomach	107.0	785	85.6	859	96.0	85.0	1.78	593	73.5	694	77.5	79.8
Lung	89.5	657	77.6	725	84.0	82.8	1.27	425	50.5	468	54.2	69.0
Kidney	93.1	681	79.0	650	76.0	85.8	1.41	472	54.5	395	46.0	75.0
Skin	93.6	688	96.2	845	103.8	101.0	2.54	850	120.0	904	111.0	95.1

* Obtained from equation 7.

† Obtained from equation 6.

‡ Average chloride 137.0 mM/kgm. serum. Average sulfocyanide 4.0 mM/kgm. serum.

another. They may be divided into two general groups, *a*, the thick muscles such as the biceps femoris or gastrocnemius having low volumes of distribution which are of similar magnitude from animal to animal, and *b*, the thin muscles having generally quite large volumes of distribution which are somewhat variable from animal to animal. In both groups, however, the sulfocyanide and chloride available water volumes of normal cats are quite similar if not identical.

II. *The size of the chloride and sulfocyanide available volumes following massive infusion of 1 per cent sodium chloride or 5 per cent glucose solution.* Water and chloride, infused as a 1 per cent sodium chloride solution, is retained for the most part in the chloride and sulfocyanide available (extracellular) water. A comparison of the volume of water retained with the increase in size of the chloride and sulfocyanide available water (table 4) shows almost complete agreement on the basis of chloride distribution and a small but consistent increase in the size of the volume of water available to sulfocyanide. Such sulfocyanide values would seem to be most reasonably explained on the hypothesis that some of the cells become permeable to this ion as a result of infusion. Skin is of interest in this connection. In the normal animal, chloride and sulfocyanide were found distributed through a volume of water equal to the total water content of the skin. Following infusion with sodium chloride solution, sulfocyanide was present in amounts indicating an average water content of 90.4 ml./100 grams whereas the total water content found was only 81.0 ml. which suggests a binding of sulfocyanide in some manner by the skin. Similar behavior seems likely to a limited extent in the case of some of the other organs.

Following the infusion of large amounts of 5 per cent glucose solution the volume of water through which sulfocyanide is distributed is more variable. A comparison between the volume of water infused and the volume of water available for the solution of sulfocyanide gives values which indicate a range of variation from no difference at all to as great a difference as 411 ml./kgm. of cat in one instance (table 4). This difference was associated with corresponding differences in behavior of the various organs. When the sulfocyanide available water is in excess of the volume of infused water retained, the individual organs show a corresponding increase in the amount of the sulfocyanide available water. It thus appears that the permeability of the cell may be increased with respect to the sulfocyanide ion as a result of massive infusions of 5 per cent glucose. On the contrary, there is no appreciable increase in the permeability of the cell to chloride as a result of glucose infusions even though the response is somewhat more variable than in those animals infused with 1 per cent sodium chloride solution. It can be concluded from chloride analyses that the glucose solution infused was confined, for the most part, to the extracellular compartment of the body.

A summary of the data obtained by analysis of the various organs following infusion is presented in table 3 a and b. There is close agreement between the volume of sulfocyanide and chloride available water for any given muscle following infusion with 1 per cent sodium chloride solution. The increase in the water content is for the most part confined to the extracellular compartment. After infusion with 5 per cent glucose solu-

tion, analysis for chloride indicates that most of the increase in water takes place in the extracellular compartment. The volumes of sulfocyanide available water are somewhat variable and for that reason the averages presented may be misleading. In one instance the injected sulfocyanide left the circulation in large amounts and in this instance the available volumes were much greater in the various muscles than the observed increases in water would indicate.

Chloride analyses of the livers of perfused animals suggest that the cells of this organ are normally impermeable to the chloride ion. Truax (13) has recently reported a comparison between the histological extra-

TABLE 4

*The effect of 5 per cent glucose and 1 per cent sodium chloride infusions on the sulfocyanide and chloride available water in cats**

	1	2	3	4	5	6	7
CAT NUMBER	(AW_s)	Water Retained	Calculated $\frac{(AW'_s)(1+2)1000}{(2+1000)}$	Observed (AW'_s)	Difference (4 − 3)	(AW'_{cl})	Difference (6 − 3)
5 per cent glucose infusion							
15	300	225	429	500	71	415	−14
18	348	182	448	490	42	530	82
20	352	131	425	836	411	459	34
21	316	164	411	387	−24	408	−3
23	389	233	504	570	66	578	74
1 per cent sodium chloride infusion							
16	232	253	386	442	56	404	18
17	327	370	512	530	18	496	−16
19	389	253	512	584	72	518	6
22	450	230	553	624	71	552	−1
24	462	264	575	657	82	557	−18

* All values in milliliters per kilogram of cat.

cellular space and the chloride available water of the liver of the white rat. He finds these to be quantitatively equal, thus giving the chloride an exclusively extracellular distribution. The calculated increase in the water in the livers of perfused cats, assuming all of the increase to be in the extracellular water, is in all instances greater than the observed increase. Approximately 40 per cent of the water taken up by this organ as a result of infusion seems to be taken into the intracellular compartment. Sulfocyanide analyses of the liver could not be performed due to interfering colors which could not be removed.

The stomach appears to contain intracellular chloride. Following the

infusion of 5 per cent glucose solution there is an average increase of 13.2 ml./100 grams in the water content of this organ whereas the chloride distribution would indicate a gain of 23.4 ml./100 grams. There must have been cellular chloride not readily available to the extracellular fluids. That the stomach readily takes up sulfocyanide in glucose infused animals is indicated by an average available volume of 108 ml. Fluid collected from the stomachs of these animals contained sulfocyanide in approximately the same concentration as the blood plasma. Following infusion of 1 per cent sodium chloride solution, chloride analysis gave an average increase in water of 31.1 ml./100 grams whereas the actual increase was only 22.4 ml./100 grams. Inasmuch as the cells of the stomach take up some chloride and sulfocyanide the estimation of the ratio of extracellular to intracellular water on the basis of the distribution of these substances is not possible. The small and large intestine behave in a manner essentially similar to the stomach.

Chloride distribution in the pancreas suggests, as above, that there is a small amount of chloride not readily available to the extracellular water. It would seem however, on the basis of the distribution of this ion, that most of the gain in water is in the extracellular compartments. Sulfocyanide analysis of the pancreas of saline infused animals gives a volume approximately one-half that for chloride distribution. This would suggest either an increase in water and chloride content of the cells or the existence of some extracellular water not readily available to the sulfocyanide ion.

An accurate analysis of available volumes of the kidney is difficult because of the uncertain amount of water, chloride and sulfocyanide in the tubules. In sodium chloride infused animals however, the increase in water content, if confined to the extracellular water, would amount to 15 ml. whereas the chloride content indicates an average increase in volume of only 6.1 ml. Inasmuch as the urine of the animals contained chloride in higher concentration than the plasma, the results cannot be explained on the basis of a salt poor fluid. There must have been an increase in the size of the cellular compartment.

The spleen did not show an increase in water as a result of infusion. This may not correctly represent the conditions in this organ inasmuch as asphyxia which causes splenic contractions was used to terminate the experiments. However, it is of interest to note that the chloride and sulfocyanide content indicates a larger amount of water than was actually found present. In the case of 5 per cent glucose infusion this was quite marked. The chloride and sulfocyanide concentration of the blood being low at the end of infusion with glucose solution, the difference between theoretical and observed values would tend to be exaggerated if these substances were not readily available to the extracellular fluids. Here, as previously suggested, there is some evidence of "binding" of these ions.

The cerebrum is of particular interest in that the sulfocyanide available water is normally very small (av. 8.7 ml./100 grams whereas the chloride available water is quite large (av. 32.5 ml./100 grams). The sulfocyanide available water is small compared to the total water available, i.e., the vascular water and cerebrospinal fluid water. The changes in the sulfocyanide content of the cerebrospinal fluid do not run parallel to changes in the composition of the plasma. The sulfocyanide concentration of this fluid is much lower than the concentration in the plasma, within the time limits of our experiments. This difference appears to result from the unique nutritive system of this organ. The low plasma chloride resulting from infusion of 5 per cent glucose solution does not materially reduce the chloride content of the cerebrospinal fluid. If a large part of the fluids of the extracellular spaces of the brain behave like the cerebrospinal fluid, values such as reported here are to be expected. The infusion of 1 per cent sodium chloride elevates the plasma chloride which in turn causes an increase in the chloride content of the cerebrospinal fluid and the cerebrum. The chloride excess of the plasma appears to diffuse readily into the chloride available water of the brain but very slowly leaves this water to pass back into the blood stream when the chloride concentration of the latter is lower. The observed decrease in the sulfocyanide available water was due, for the most part, to the decrease in the sulfocyanide concentration of the blood plasma resulting from infusion. The sulfocyanide which had passed into the cerebrospinal fluid before infusion with 1 per cent sodium chloride is little influenced by the changes in the sulfocyanide concentration of the blood plasma resulting from infusion.

Reference has previously been made to the skin. In a few of the perfusion experiments, edematous subcutaneous tissue was dissected away and was separately analyzed. This invariably gave a value higher than the epithelial layers overlying it. In one glucose infused animal the skin was found to have 69.7 ml./100 grams of chloride available water whereas analysis of the subcutaneous tissues gave a value of 102 ml./100 grams. Sodium chloride infusion resulted in skin values of 91.0 ml. and 95 ml. respectively for sulfocyanide and chloride available water whereas analysis of edematous subcutaneous tissues gave values of 125 ml. and 120 ml./100 grams on the basis of sulfocyanide and chloride content. The high skin values obtained would appear to result from the "binding" of chloride and sulfocyanide by their subcutaneous tissues rather than by the epithelial elements.

Discussion. Recent studies of the total chloride (2) and sulfocyanide (1) available volumes of various species of animals give a normal range of 25 to 45 per cent of the body weight. Our observations on the cat gives similar volumes of distribution for these two substances. Comparison

of the sulfocyanide and chloride available volumes in the same animal indicates a slightly greater available volume for the latter. This difference we believe to be due to "bound" chloride in some tissues. This possibility is suggested by the work of Amberson, Nash, Mulder and Binns (3) and more recently by the investigations of Manery and Hastings (12).

Several investigators have attempted to determine the extracellular-intracellular water ratio for some organs of the animal body (Harrison, Darrow and Yannet (2) chloride available volumes of the rabbit, dog and monkey; Wallace and Brodie (11) the chloride and bromide available volumes of the dog and the chloride, bromide and sulfocyanide available volumes of the various parts of the central nervous system of this animal; Manery and Hastings (12) the sodium and chloride available volumes of the rat and rabbit). The available volumes reported for any organ agree in a general way with the values here reported for the normal cat and suggest a similar distribution of these ions in the mammals studied. In some instances values have been given for skeletal muscle or intestine without specifying the particular muscle or part of the intestine which was taken for analysis. We have found the amount of chloride and accordingly the chloride available water of muscles to vary widely. The chloride available water of the duodenum is, on the average, less than that of the colon. In the uninfused cat there is reasonably good agreement between the volumes of water available to chloride and sulfocyanide in skeletal muscle. A comparison of the values for these two substances in other organs shows similar magnitudes of distribution in all organs except the cerebrum. The investigations of Wallace and Brodie (11) suggest that a similar difference in available water would be found for the whole central nervous system.

A comparison between the volume of water retained and the increase in the amount of the chloride available water following infusion of 5 per cent glucose and 1 per cent sodium chloride suggests that most of the fluid infused is retained in the extracellular compartment but analysis of individual organs shows that this is not strictly the case. The stomach takes up chloride to a greater extent than water in the case of infusion with 1 per cent sodium chloride solution and does not readily lose chloride after infusion with 5 per cent glucose solution. The cerebrum readily gains chloride as a result of 1 per cent sodium chloride infusion and very slowly loses chloride when the concentration in the blood is lowered by infusion with 5 per cent glucose solution. In neither case does the brain gain appreciable amounts of water. The skin appears to contain salt and water in comparable amounts but separate analysis of the epithelial portion and the subcutaneous tissue suggests that the latter is most important in binding

both chlorides and sulfocyanide, containing these substances in exceedingly large amounts. Manery and Hastings (12) have likewise found that connective tissues may "bind" large amounts of sodium and chloride.

Our data suggest that the thick muscles and possibly the liver most nearly have a strictly extracellular distribution of chloride and sulfocyanide and that infusion of 5 per cent glucose may alter the distribution of the latter ion.

SUMMARY

1. Chloride and sulfocyanide are distributed through a volume of fluid greater than that contained in the extracellular compartment.

2. These substances are normally limited to the extracellular spaces of skeletal muscle. Chlorides appear to be confined to the extracellular compartment of the liver.

3. There is some intracellular chloride in the stomach, small and large intestine, spleen and pancreas.

4. Chloride and sulfocyanide are distributed through a volume of fluid approximately equal to the total water content of the skin.

5. Infused 5 per cent glucose or 1 per cent sodium chloride solution is retained for the most part in the extracellular compartment.

6. Infusion of 5 per cent glucose may greatly increase the permeability of the cells of certain tissues and organs to sulfocyanide.

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POST-TETANIC POTENTIATION AND SUPPRESSION IN MUSCLE

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The literature concerning the nature of the augmenting or potentiating effect of an indirect tetanus on the response of a muscle to subsequent single nerve volleys is conflicting. Guttman *et al.* (1) found no potentiated response after a direct tetanus in the curarized frog muscle while Brown and v. Euler (2) found it in cat muscle. The magnitude of the muscle action-potential accompanying the potentiated twitch is less than that of the last twitch before the tetanus in the normal cat (2) and in the normal fowl (4), whereas in the partially curarized cat muscle it is greater (2). Brown and v. Euler think that in the normal cat muscle the potentiated twitch is the result of increased tension in each muscle fiber and that this increase is due to an accumulation of potassium ions outside of the muscle fiber as a result of tetanization. On the other hand, they believe (2) that in the partially curarized cat muscle the potentiated twitch is due to the response of more muscle fibers to the nerve volley and that this is due to a mobilization of potassium at the myoneural junction. In normal fowl muscle the potentiated twitch is likewise supposed (4) to be due to an increased number of muscle fibers responding, but this increase is not ascribed to a mobilization of potassium. Rosenblueth and Morison (3) and Feng *et al.* (5) explain potentiation in the normal cat as due to a mobilization of potassium at the myoneural junction, but Feng (6) believes that in the toad mobilization of acetylcholine brings it about, while Guttman *et al.* (1) think that it is due either to acetylcholine or to adrenine.

It is the object of this paper to attempt to resolve this conflict of interpretations and more particularly to answer the questions of Bronk and Brink concerning post-tetanic potentiation (7, p. 402): "Why a sympathetic ganglion should resemble a partially curarized muscle and whether it is indeed the same type of mechanism have not yet been determined." This attempt involves a study of the muscle and nerve action-potentials simultaneously and a reëxamination of potentiation during curarization and in the presence of potassium.

METHODS. The sciatic nerve and gastrocnemius muscle of *Rana pipiens* were dissected out in the usual way and placed in a beaker of amphibian Ringer's solution

for from ½ to 2 hours before using. Sometimes the preparations were kept 24 hours in the refrigerator at 3 to 4°C. before using; these preparations were usually less excitable than fresh preparations. The action-potentials of the nerve and the response to direct stimulation in the latter preparations seemed normal, so that apparently the myoneural junction deteriorates more quickly than either the muscle or nerve fibers.

The preparations were then placed in a modified Keith Lucas bath. The modification consisted of a hard rubber block which replaced the end of the bath opposite the lever attachment. Five holes, 2 to 3 mm. in diameter and 6 to 7 mm. deep, were drilled along the block. The holes were connected by a trough 2 mm. wide and 3 mm. deep. The nerve was placed in the trough which was packed with white vaseline and the holes were filled with Ringer's solution. The knee joint was fixed and the tendon attached to the lever which was free weighted and which magnified 8 times. Mechanical records were made on smoked paper fastened to a spring-driven kymograph.

Chlorided silver electrodes attached to plugs in a bakelite panel were lowered into the holes in the block. The first two delivered make induction shocks from a coreless coil specially wound. This coil was driven by a special tube circuit which was synchronized with the sweep circuit of the oscillograph. For direct stimulation of the muscle a thyratron circuit was used without any induction coil. Both circuits could deliver shocks anywhere from 1 per second to 450 per second. The third electrode was connected to ground which got rid of most of the shock escape. The last two electrodes were connected to a 5-stage, capacity-coupled amplifier. When the muscle action-potentials were also studied, an insulated wire with bared tip was inserted into the belly of the muscle and connected to the proximal pick-up electrode on the nerve. There was no confusion of the action-potential of the muscle and that of the nerve on the screen of the DuMont type cathode ray oscillograph due to conduction time and junctional delay. Sometimes two leads from the muscle were used, and then the action-potential was recorded on a Davis-Grass encephalograph.

A celluloid plate with ruled lines on it, placed over the face of the oscillograph, served to measure the heights of the action-potentials which were recorded in arbitrary units as seen. The potentials recorded by the encephalograph were measured with calipers and recorded in millimeters, as were the tracings of the mechanical response which were recorded isotonically.

Curare was given *ad libitum* until curarization was complete as was evidenced by no response to an indirect tetanus of 30 per sec. Lack of response to single shocks does not indicate complete curarization. Potassium was added to the medium (40 cc.) bathing the muscle in the form of a 5 per cent solution of potassium chloride. Sometimes the 5 per cent solution was diluted with Ringer's solution before using. The solution was added dropwise near the knee-joint. Each drop was about 0.05 cc. The whole solution was then drained off and replaced by fresh Ringer's solution. Recovery from the effects of the potassium was quite rapid when small quantities were used. With very large quantities, the muscle sometimes remained in permanent contracture.

RESULTS. *The action potential of the potentiated twitch.* Table 1 and figure 1 show that the muscle action-potential accompanying a mechanically potentiated twitch is greater than that accompanying the last twitch before the potentiating tetanus.

Table 1 reveals two other facts that are important for the understanding

of post-tetanic potentiation. First, the twitch-tension of the first twitch following the conditioning tetanus (*e.g.*, after tetani 2 and 3) may be suppressed although its accompanying action-potential is increased in comparison with the values before the tetanus. This indicates that, in spite of greater excitability of the motor end plates, the muscle fiber is

TABLE 1

The relation of the twitch-tension (T) in millimeters to the magnitude of the muscle action-potential (M) in millimeters as recorded by the electroencephalograph

Single shocks, 1 per second; conditioning tetani, 30 per second for 5 seconds.

M	T		M	T		M	T		M	T
			7.5	16.4		5.4	4.1		5.5	4.0
			7.8	16.7		7.0	10.0		6.4	7.5
			7.4	14.6		8.0	10.8		6.3	6.0
			7.4	13.9		7.6	8.9		5.6	4.9
			7.1	12.4		6.8	8.2		5.1	4.5
		Tetanus 1	6.4	11.0	Tetanus 2	5.3	6.1	Tetanus 3	4.2	3.6
			5.9	9.8		5.4	5.5		3.6	3.2
			5.3	9.5		5.2	4.7		3.5	2.5
			5.4	8.4						
6.9	8.1		5.4	7.0						
6.5	7.5									
6.9	7.8		4.9	4.9		4.0	1.7		2.9	1.5
6.4	7.3		4.9	4.8		4.0	1.7			

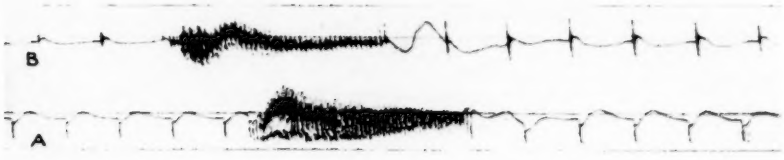

Fig. 1. Single shocks, 1 per sec.; conditioning tetani, 30 per sec. for 5 sec. Potentials recorded with encephalograph.

A. Monopolar lead, belly of muscle—distant part of bath. Untreated muscle not greatly fatigued.

B. Bipolar leads, belly-tendon. Muscle had previously been treated with potassium, but the solution had been drained and its effect had passed off 5 min. before.

unable for a short time mechanically to respond. And second, after a tetanus the magnitude of the first muscle action-potential is regularly less than the magnitude of the second. A simultaneous examination of the accompanying nerve action-potentials shows that after the conditioning tetanus the number of nerve fibers responding to the single shocks is

reduced; this accounts for the difference between the first two muscle action-potentials.

Suppression. If the conditioning tetanus is of relatively high frequency and long duration, or if the strength of the test-shock is relatively low, so few nerve fibers will respond to the test-shock that the electrical and mechanical response is less than before the conditioning tetanus or it may disappear entirely. This is called post-tetanic suppression or junctional inhibition and it is sometimes attributed to the depressant effect of a chemical mediator which has accumulated in large quantities (*e.g.*, 5, 6). However, as in figure 2, every case of "suppression" except when the muscle is completely fatigued (as shown by absence of response to a direct stimulation or failure to recover with rest)

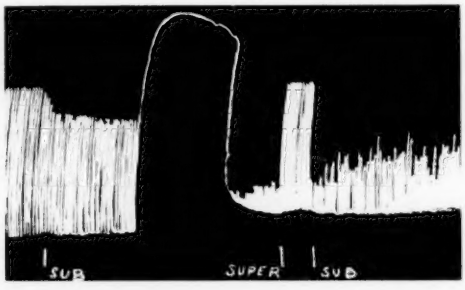

Fig. 2. Fresh, untreated preparation. Single shocks, 1 per sec.; conditioning tetanus, 30 per sec. for 30 sec. At *SUB*, shocks were made submaximal. After the tetanus the mechanical response was reduced 85 per cent, and the nerve action-potentials 70 per cent. At *SUPER*, the shocks were made supramaximal, the mechanical response and nerve action-potential becoming normal. At *SUB*, the shocks were made submaximal again. The mechanical response increased with the increase in the nerve action-potential.

can be overcome by raising the strength of the testing shock delivered to the nerve until the oscillograph shows that as many nerve fibers are responding as did before the conditioning tetanus.

A necessary condition for obtaining post-tetanic potentiation is that the testing shocks must be strongly supramaximal, for if the shocks are relatively weak and the conditioning tetani fairly fast and long, variations, up to and including extinction, appear in the picture of potentiation. In each case, oscillographic examination of the nerve fibers showed that this was due to the failure of some or all of the nerve fibers to respond on account of the raised threshold accompanying the period of positive after-potential which follows the tetanus (8). This is undoubtedly also the explanation of results obtained by Guttman *et al.* (1) and Brown and v.

Euler (2); the latter authors merely say that it is "an additional factor" coming into play.

The effect of curare on potentiation. Brown (2) claims that it is possible to obtain potentiation of the same order of magnitude in the curarized preparation as in the normal. Guttman *et al.* (1), on the other hand, reported that they could not. A confirmation of one or the other of these reports is of paramount importance in deciding whether post-tetanic

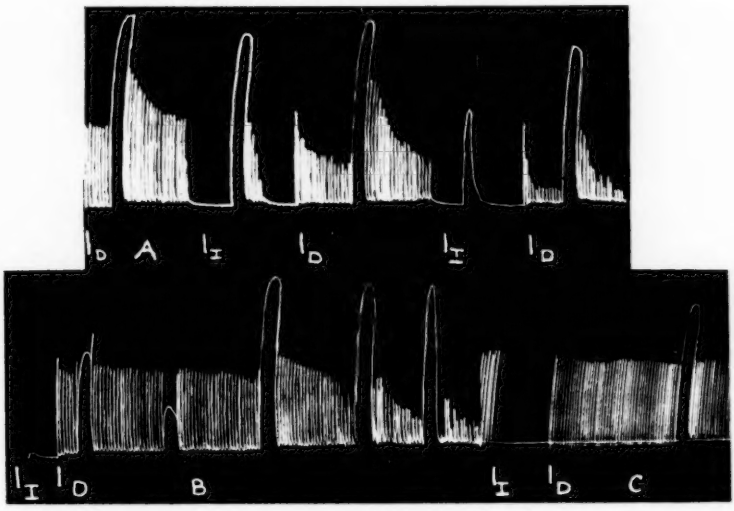

Fig. 3. Curare administered before *A*. Until the beginning of *A*, the same amount of potentiation was obtained by indirect stimulation as by direct. Single shocks, 1 per sec.; conditioning tetani, 30 per sec. for 5 sec.

A. *D* stands for direct stimulation, and *I* for indirect stimulation. *B*. For the third and subsequent direct tetani the voltage was doubled during the tetani. *C*. Between *B* and *C* there was a minute rest. The voltage was raised for the direct tetanus.

potentiation is a function of the myoneural junction or of the the muscle fiber.

When an indirect tetanus of 30 per sec. for 5 sec. still produces a mechanical response of the muscle, figure 3A, that is, when it has a decurarizing action, it is possible to obtain post-tetanic potentiation by direct stimulation, and this potentiation is of a magnitude comparable with that obtained by indirect stimulation. Another point to notice in figure 3A is that although the muscle responded to indirect tetani it did not respond to single shocks before the tetanus; it did afterwards due to the

decurarizing effect of the tetanus. This direct potentiation was undoubtedly due to the effect of the direct stimuli on the nerve endings or motor end-plates of the muscle, for when, as in figure 3B, there was finally no decurarizing action of a supramaximal indirect tetanus, no potentiation was obtained with direct stimulation.

In some cases it was possible to obtain an augmentation of the response to single direct shocks after a tetanus but only under certain conditions such as raising the voltage during the conditioning tetanus or rest. However, this augmentation did not always appear even when these conditions were fulfilled, *cf.* figure 3C where the preparation was both rested and the voltage of the tetanus raised with negative results. When the augmentation did appear, its magnitude never exceeded 25 per cent and it rarely lasted for more than 10 sec. This is very much below the normal range

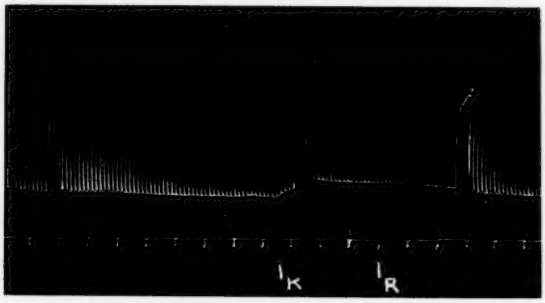

Fig. 4. Fatigued preparation, previously untreated. Single shocks, 1 per sec.; conditioning tetani, 30 per sec. for 5 sec. At *K*, the concentration of potassium in the bath (50 cc.) was raised 50 times. At *R*, this solution was drained and replaced by fresh Ringer's solution.

of increase in tension (500–1000 per cent) and of the persistence of the increase (20–60 sec.) obtained in untreated preparations by stimulation of the nerve.

If excitability of the motor end-plates was depressed by fatigue instead of by curare, the very same results were obtained with direct stimulation, namely, at any one time the magnitude of potentiation was the same for both direct and indirect stimulation, and when the myoneural junctions failed to respond to indirect stimulation, no potentiation except under the conditions described above was obtained with direct stimulation. The only difference between fatigue and curare was that the effect appeared more quickly with curare.

The effect of potassium on post-tetanic potentiation. Figure 4 shows that when the concentration of potassium was raised 50 times in the medium bathing the muscle, potentiation could still be obtained after a condi-

tioning tetanus, although this potentiation was less in magnitude and duration than those before and after the administration of the potassium. By adding the potassium slowly, only a slight contracture was produced which did not obscure the loss of irritability. When added in this way, the potassium had a curariform effect, but nevertheless, as figure 4 shows, the tetanus still had a slight decurarizing effect.

When potassium was added suddenly only two effects, if any, on potentiation, irrespective of the amount, were ever found: first, when moderate amounts were given there was a decrease in magnitude and duration of the potentiation. And second, if contracture was produced, as it was when large amounts of potassium were given, the potentiated twitches rose above the level of the contracture in such a way that from the point of view of the *crests* of the twitches, an ordinary potentiation picture was

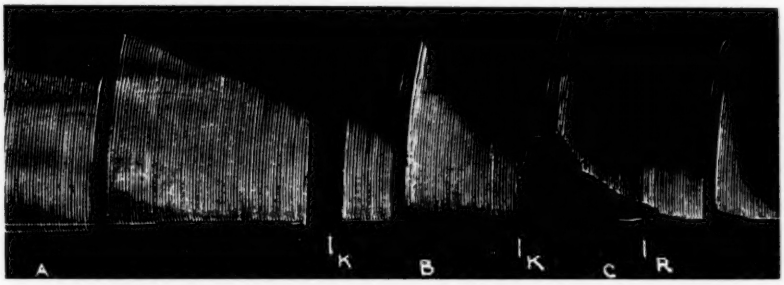

Fig. 5. Fresh, untreated preparation. Single shocks, 1 per sec.; conditioning tetani, 30 per sec. for 5 sec.

A. Normal potentiation in slightly fatigued preparation. *B*. Between *A* and *B*, 0.1 cc. of a 5 per cent solution of KCl was added to the bath (50 cc.). Potentiation picture is typical of middle fatigue. *C*. At *K*, 1.0 cc. of a 5 per cent solution of KCl was added. At *R*, this was drained off and replaced by fresh Ringer's solution.

obtained, figure 5B and 5C, although when the *absolute height* of the twitch above the contracture level was measured, there was no potentiation.

All the experiments that we have done show either a slight decrease in magnitude and duration of potentiation, or none as in figure 5B, or a contracture when potassium is added, but there was never any enhancement of the magnitude or duration of potentiation on the addition of potassium. Brown and v. Euler (2) also found that potentiation was never affected when potassium was injected into the artery during the conditioning tetanus (intact cat preparation).

Table 2 shows the effect of potassium on the twitch-tension and muscle action-potential. The twitch-tension fell gradually until a suppression of 17 per cent developed; about the 70th twitch the tension was again normal. On the other hand, the muscle action-potential fell to about 75

per cent of its original value, and this fall bore no relation to the decrease and subsequent increase in tension. With smaller doses potentiation of the twitches instead of suppression was obtained, confirming Wilson and Wright (12).

Discussion. If a muscle is partially fatigued by indirect stimulation with single maximal volleys or artificially fatigued by partial curarization, a rise in threshold of some of the motor end-plates of the muscle fibers results so that these no longer respond to single volleys. In axons Erlanger and Blair (19) have termed an analogous appearance of subnormal ex-

TABLE 2

The effect of potassium on the twitch-tension (T) in millimeters and action-potential of the muscle (M) in millimeters as recorded by the electroencephalograph

Rate of stimulation, 1 per second; 0.5 cc. of a 5 per cent solution of KCl was added.

M	T	REMARKS
8.5	40.3	
8.3	39.5	
8.2	45.1	Potassium added
6.9	39.5	
6.8	37.5	
5.4	37.0	
4.5	34.3	
4.1	35.0	
3.9	34.3	
3.7	33.7	
3.5	33.2	
2.5	33.4	
2.4	35.0	Potassium drained Fresh Ringer's solution added
70 unmeasured twitches		
1.9	39.5	
1.9	35.8	
1.7	40.0	

citability "disbandment." There is reason to suppose that the two phenomena are similar, because if a succession of nervous impulses arrives at these disbanded end-plates closely enough together, as during a tetanus, the muscle fiber will finally contract. The reason for this is that each blocked impulse raises the excitability of the end-plate until one impulse finally becomes effective. In axons this process of facilitation by a train of subthreshold stimuli is called "recruitment," and it was demonstrated at an anode block by Erlanger and Blair (19) and at the stimulating electrodes during a period of after-positivity with accompanying subnormal excitability by Gasser (20). Evidence is as yet lacking to show that the

motor end-plate is in the same physico-chemical state when fatigued as is the axon during the conditions just mentioned.

After a disbanded muscle fiber has been recruited in this manner during a tetanus, its enhanced excitability may persist for from 20 sec. to 1 min. after the end of the tetanus—in frog muscle at least. Now although this phenomenon has no analogy in axons, it does have one in autonomic synapses. Larrabee and Bronk (11) showed that in the stellate ganglion the degree and duration of facilitation is increased by repetitive stimulation; for example, a short tetanus increased the response to a submaximal volley 4 to 5 times, and this enhanced excitability persisted for about 60 sec.

Thus potentiation involves recruitment of muscle fibers from a reservoir of idle muscle fibers set up by fatigue of the end-plates or, artificially, by partial curarization; this recruitment occurs during tetanic stimulation. Potentiation also involves the persistence of the enhanced excitability of the end-plates for a relatively long period after potentiation is over.

This view is consistent with the fact that potentiation cannot be obtained by direct stimulation when the muscle is completely curarized (*cf.* also 1). That is, potentiation is a myoneural process, not a function of the muscle fiber itself, as Brown (2) claims. His results were probably due to incomplete curarization as he did not use tetani to test for curarization (*cf.* 3). It is also consistent with the fact that the results of alternate direct and indirect stimulation during normal fatiguing of the myoneural junction were identical with those obtained with curare. This indicates that curarization is an artificial "fatiguing" of the myoneural junction (*cf.* 10) and also that post-tetanic potentiation is the identical phenomenon in both circumstances. Potentiation, it is known (1), cannot be obtained in the normal frog muscle when unfatigued; it can be obtained in the fresh fowl muscle where some end-plates normally never respond to a maximal single volley. Brown (4) says that potentiation in the latter case is like that of the partially curarized cat muscle where the increased tension is due to an increased number of fibers responding to the nerve volley.

Still further support is found in the fact that in the frog at least the muscle action-potential accompanying the potentiated twitch is increased while that of the nerve is decreased when compared with those accompanying the last twitch before the conditioning tetanus. This means that, although fewer motor units respond to the testing shock after the tetanus due to the raised threshold of the nerve fibers which accompanies the positive after-potential produced by the tetanus (8), more muscle fibers are excited to contract within the motor units that do respond, thus producing an increased twitch tension.

This is confirmed by the direct findings of Asmussen (9) who found that not all the muscle fibers in a motor unit respond to a single volley;

during a tetanus, however, the number of active end-plates increased steadily until fatigue set in. The fatigue may be end-plate fatigue (the "all-or-none" response) or fiber fatigue (the "graded" response); in the isolated nerve-muscle preparation he observed that end-plate fatigue intervened first.

Chemical versus electrical theory of potentiation. Chemical. Because of the rapidly destructive action of choline esterase, acetylcholine cannot be the cause of relatively long-enduring potentiation (2). Instead a mobilization of potassium at the myoneural junction is believed to cause potentiation because the effects of injected potassium on single twitches mimic the effects of tetani (2, 3, 5). There are several facts, however, that militate against this. Fenn (13) concluded that either no potassium left cat nerve fibers during tetanization or else that it returned so rapidly that his methods of analysis were unable to detect its escape; the tetani he used were much faster and longer than ordinary potentiating tetani. We found, and Brown (2) did also, that no combination of tetanus and administered potassium had any effect on the duration and magnitude of post-tetanic potentiation. An excess of potassium suppresses the mechanical response of single twitches; a prolonged, high frequency tetanus, however, exerts its effect on the nerve and not at the myoneural junction (*cf.* fig. 2). In large doses potassium seems to have a curariform effect, yet a conditioning tetanus still evokes potentiation (*cf.* fig. 4). If potassium were mobilized at the myoneural junction during the tetanus, a still further depression of excitability would be expected. Finally, we agree with Brown's (15) conclusion that injected potassium exerts its augmenting effect on single twitches by acting on the contractile mechanism of the muscle fiber ("graded" response) and not at the myoneural junction, the site of the potentiating effect of a tetanus.

Electrical. Erlanger (16, 19) points out the similarity of the time-course of changes in excitability after a subthreshold stimulus at the muscle synapse (*cf.* 10), at a block in the axon, at the stimulating electrodes on axons, and at the synapses of motoneurons. This leads him to believe that "the common denominator obviously is electrical," that at an artificial synapse in an axon, and also by inference, at a natural synapse, the action-potential spike acts as an electrical stimulus to the tissue beyond (19), and that the chemical theory of transmission does not explain the facts (16).

That this is the explanation of the recruitment phase of potentiation is supported by the fact that the times for the latent addition (10) of two subthreshold impulses at the frog muscle synapse (optimum, 4 msec.; maximum, 70 msec.) fit fairly well with potentiating frequencies, for example, the lower limiting frequency for the frog is around 10 per sec. (1). The optimal frequency, however, is given as 30 per sec.; this dis-

crepancy is due to the fact that higher frequencies produce a subnormal excitability in the nerve, which prevents the appearance of potentiation (*cf.* fig. 2). With strong test shocks, however, we have observed potentiation with tetani of 100 per sec., and Feng *et al.* (5) reported it with tetani of 240 per sec. Katz (14) showed that a subthreshold cathodal current to the region of the myoneural junction could facilitate a blocked nervous impulse and that the facilitation curve was the same as that for latent addition. This facilitation is obtainable "*after the withdrawal* of a direct electrical stimulus," and, therefore, in his opinion, it is due to the subsidence of the local excitatory effect of the stimulus and not of a transmitter substance. The rate of subsidence of the local excitatory effect which outlasts the nervous impulse is characteristic of the alpha excitability of muscle (21). The local excitatory effect can be observed at the myoneural junction as it produces electrical changes whose time-course differs from those associated with the propagated disturbance (17). There is no evidence that this local effect is due either to acetylcholine (18) or to potassium (15). Nor is there any evidence that the prolonged enhanced excitability lasting as long as 1 min. following the tetanus is due to a persistence of potassium at the myoneural junction.

The question of Bronk and Brink, quoted at the beginning of this paper, may be answered by saying that potentiation in the normal sympathetic ganglion resembles potentiation in the normal muscle as well as the partially curarized, and that, further, it seems likely that the mechanism is the same in both.

SUMMARY

The problem of post-tetanic potentiations was studied in the isolated frog sciatic-gastrocnemius preparation. The muscle and nerve action-potentials and the twitch-tension were observed simultaneously. Particular attention was paid to the effect of curare and potassium on potentiation.

Potentiation was not obtained in the completely curarized preparation by direct stimulation. It was obtained in the partly curarized preparation. Normal fatiguing of the myoneural junction gave the same results as curare did.

Potentiation was obtained after the administration of depressing doses of potassium. Potassium never enhanced the potentiating effect of tetani.

The muscle action-potential accompanying the potentiated twitch was greater than that accompanying the twitch preceding the conditioning tetanus. The nerve action-potential after the tetanus was usually decreased.

Suppression of the muscle response after a prolonged tetanus was due to a decrease in the number of nerve fibers responding to the test-shock.

This suppression could be overcome by raising the strength of the test shock.

These results were discussed in the light of current explanations of potentiation which were found to be inadequate. It was concluded that potentiation was the same in the partly fatigued frog, partially curarized mammal, and normal fowl muscle and that it was due to the facilitating effect of blocked nerve impulses and an enhanced local excitatory state persisting at the motor end-plates after tetanization. It was suggested that since others have shown the inadequacy of the chemical theory of neuromuscular transmission in explaining these events, a chemical explanation of post-tetanic potentiation is also inadequate.

We would like to thank Prof. H. S. Liddell for advice and encouragement during this research.

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ANOXIC EFFECTS OF HIGH OXYGEN PRESSURE ON SMOOTH MUSCLE[1, 2]

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Exposure of animals to oxygen at high pressure induces changes which involve smooth muscle tissue. The reaction in this tissue might conceivably arise as a result of a disturbed carbon dioxide transportation by the blood or as a result of a more direct effect of high oxygen on tissue. In either case the exact mode of action could be one or several of a possible great variety. The experiments herein reported were undertaken then with a number of questions in mind: First, are the effects of high oxygen on smooth muscle necessarily dependent on central nervous or hematogenous connections? Second, if these effects can result from a peripheral influence of high oxygen is such influence mediated through intrinsic nerve endings, or are they due to a "direct" action on the effector itself? Third, what is the nature of this direct action?

The smooth muscle investigated was that obtained from the gastrointestinal tract of a rabbit freshly killed by a postcephalic blow by hand. The gut was removed, its lumen gently rinsed, and suspended in a bath of Tyrode solution within a pressure chamber. This bath was continuously bubbled with pure oxygen saturated with water vapor and warmed to 37.5°C. An isotonic optical lever was used to record the rhythmic contractions and changes in tonus. Longitudinal duodenal and pyloric sphincter tissues were studied.

RESULTS AND DISCUSSION. *Duodenum.* Exposure of longitudinal duodenal muscle to oxygen at 75 pounds' pressure resulted in a progressive decrease in tonus. This decrease became evident in some experiments within 20 minutes and invariably was very pronounced in all, one hour after cessation of compression. The regular rhythmic contractions, which at atmospheric pressure recurred at a frequency of 14 per minute, were replaced by contractions of irregular amplitude. Frequently, and especially so in the longer exposures, the amplitude of the rhythmic contractions diminished so that for short intervals there was complete cessation of rhythmic contractions. Not only was the amplitude diminished by high

[1] These experiments were supported by a grant from the Rockefeller Foundation.

[2] Preliminary report: Am. J. Physiol., Proc. 126: p. 437, 1939.

oxygen but also the frequency was decreased. In the more prolonged exposures there appeared a gradual development of recurrent spasmodic changes of tonus. These recurrent tonic changes interspersed by periods of relative quiescence increased in severity as the pressure was maintained and continued on into the early stages of decompression.

Decompression to atmospheric pressure was invariably followed by a reversal of these compression effects. The long spasmodic waves in tonus gradually gave way to a steady tonus level which in many instances was appreciably higher than that obtaining preceding compression; the frequency of rhythmic contractions returned to normal; the amplitude became regular, but with few exceptions was smaller than that preceding compression. A second exposure of the tissue to high oxygen was attended by an earlier onset of effects essentially similar to those recorded in the first exposure; the recovery on the second decompression, however,

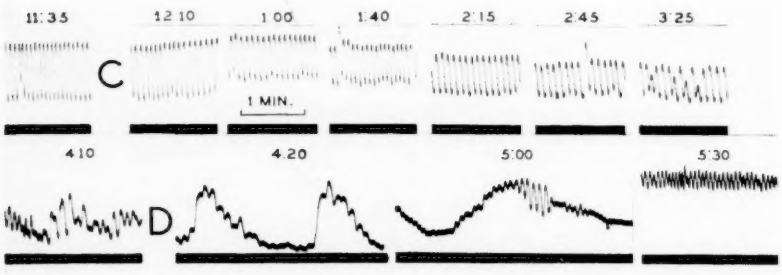

Fig. 1. The effects of oxygen at high pressure on a longitudinal duodenal strip (rabbit) in Tyrode solution. Time of day is indicated above each recording. *C* indicates compression of oxygen to 75 pounds' pressure; *D* indicates point of decompression to atmospheric pressure.

was less complete than that following the first exposure. Typical results derived from an experiment in which a longitudinal duodenal strip was employed are shown in figure 1.

The tonus changes which occurred in a control duodenal strip exposed to oxygen at atmospheric pressure and those induced in a similar preparation by exposure to high oxygen pressure are contrasted in figure 2, part 1. The earlier onset of effects in successive exposures as well as the slower and less complete recovery on decompression from successive exposures are well illustrated in this graph. The overshooting of the precompression tonus level on decompression (see fig. 1) appears from this graph to be a rapid resumption of the progressively increasing tonus obtaining before the first compression. This interpretation is supported by the practical coincidence of the control curve with that of the test. The changes in amplitude of the rhythmic contractions of these same control and test preparations,

the tonus changes of which are shown in part 1, are plotted in part 2 of figure 2.

These results clearly indicate that oxygen at high pressure can profoundly affect isolated smooth muscle and that the reaction of this tissue in animals exposed to high oxygen pressure is not necessarily dependent upon nervous connections to the central nervous system, or upon hematogenous connections to remote sources of humoral substances such as adrenalin, or

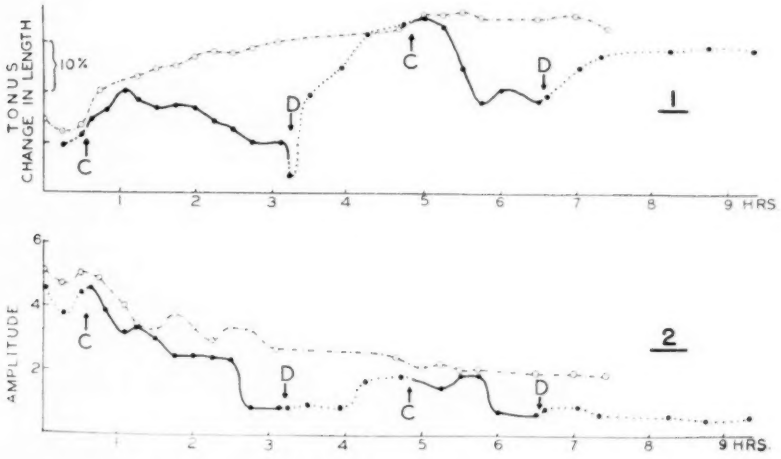

Fig. 2. *Part 1.* In the upper curve (dot and dash) the change in tonus (relaxed length) of a strip of rabbit duodenum exposed to oxygen at atmospheric pressure in Tyrode solution is plotted against time in hours on the abscissa.

In the lower curve the changes in tonus induced in a similar preparation (from the same animal) by exposure to oxygen at 75 pounds' pressure. The dotted line represents tonus during exposure to oxygen at atmospheric pressure; the solid line represents tonus during exposure to oxygen at 75 pounds' pressure. *C* and *D* indicate the points of compression and decompression respectively; *C'* and *D'* the same for a second such exposure.

Part 2. These curves show the changes in amplitude of the rhythmic contractions of the preparations whose tonus changes are shown in part 1.

on an upset in the carriage of carbon dioxide by the blood. It is recognized, however, that in an animal poisoned by high oxygen, the mediation of some effects over nervous and hematogenous connections to peripheral structures does occur as a result of the hypercapnia which arises from a disturbed function of hemoglobin in carbon dioxide transportation.

It is conceivable that the response of isolated smooth muscle to oxygen at high pressure might be due to some action of oxygen on intrinsic nerve plexuses, nerve endings or the effector cells themselves. The precise

evaluation of the possible contributions by these structures to the final response is a difficult problem especially if "there is no drug which has on plain muscle an action clearly comparable with that of curare on striated muscle in paralyzing the nerve endings" (Evans, 1926). However that may be, if intrinsic nerve plexuses and endings do play an intermediate rôle in the production of the high oxygen effects we should expect that such effects would simulate those elicited by neurohumoral substances such as adrenalin and acetylcholine. Experiments were therefore performed in which various dilutions of adrenalin and of acetylcholine were added to the Tyrode baths of fresh duodenal tissue. In each instance the response of the muscle to either of these substances was so very dissimilar from the high oxygen response as to indicate that if the intrinsic nerves or their endings are affected by high oxygen they do not mediate any effects via these neurohumoral substances.

Pyloric sphincter. The possibility that the endings of the sympathetic and parasympathetic supply to the pylorus may not always have motor and inhibitory functions identical with the corresponding endings in the duodenum, suggested that further information concerning the possible involvement of intrinsic nerves in oxygen poisoning of isolated tissue might be uncovered by a study of the effects of oxygen at high pressure on pyloric sphincter muscle. Experiments were therefore carried out in which the procedure was essentially the same as that employed in the experiments on the duodenal tissue. It was found that raising the oxygen pressure from atmospheric to 75 pounds induced a fall of tonus and that decompression to atmospheric pressure was followed by a return of tonus to the precompression level, effects similar to those recorded from duodenal strips. Typical results of these pyloric sphincter experiments are shown in figure 3 (I, II, III) where tonus (relaxed length) is plotted against time in minutes. Similar treatment with air failed to alter the tonus and so rules out the possibility that the effects produced by oxygen at high pressures are due to pressure alone. A subsequent exposure of the same tissue to oxygen at 75 pounds' pressure gave effects (graph III) essentially similar to those induced by the first exposure to high oxygen. If then it be assumed that the functions of nerve endings in the pylorus are not identical with those in the duodenum, the similarity of action of oxygen at high pressure on these two tissues would indicate a non-involvement of the intrinsic endings.

If we assume that the parasympathetic endings are inhibitory to the pyloric sphincter and that this inhibitory influence is accomplished through a liberation of acetylcholine the question arises: Does the decrease in tonus of pyloric sphincter muscle which occurs during its exposure to high oxygen pressure depend upon a stimulation of parasympathetic nerve endings and a release of acetylcholine? So in another attempt to answer this question of intrinsic nerve ending involvement in oxygen poisoning, atropine was

used. Now although atropine might not be expected to completely paralyze parasympathetic action (due perhaps to intracellular nerve endings, Cannon and Rosenblueth, 1937) one may justifiably expect that it would at least diminish extracellular parasympathetic influence. Several experiments were therefore performed in which the Tyrode bath of sphincter preparations was atropinized previous to their exposure to high oxygen pressure. Such treatment did not alter the effectiveness of high oxygen in decreasing the tonus. Similar atropinization of longitudinal duodenal strips likewise did not alter the response of this tissue to high oxygen

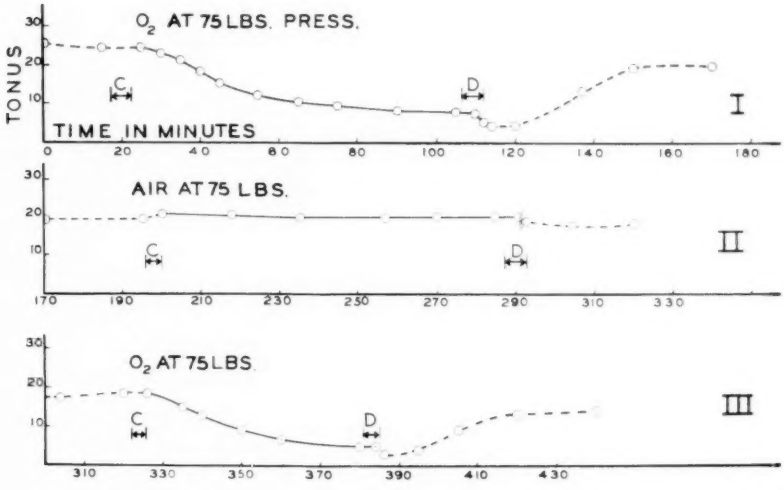

Fig. 3. Pyloric sphincter muscle. I. Tonus change as elicited by oxygen at 75 pounds' pressure. II. The tonus of the same tissue exposed to air at 75 pounds' pressure. III. Tonus change in the same tissue as a result of a second exposure to oxygen at 75 pounds' pressure. The duration of exposure to increased pressure of 75 pounds is indicated by the solid line portion of each curve. The broken line indicates exposure to oxygen (or air II) at atmospheric pressure. *C* and *D* indicate compression and decompression respectively.

pressure. While the results of these atropinization experiments do not offer conclusive evidence they do lend support to the belief that parasympathetic endings or parasympathomimetic substances are not essential intermediaries in the oxygen poisoning of isolated smooth muscle.

Cyanide, low oxygen. In oxygen poisoning of the circulated animal there occurs a decrease in oxygen consumption (Bert, 1878; Hill and Macleod, 1903; Bean, 1931). This is due in part to an increased acidity arising from a disturbed carbon dioxide transportation but apparently there is a second factor contributing to the lowered oxygen consumption, *viz.*, a poisoning

of respiratory enzymes by the high oxygen pressure. One would expect this enzyme poisoning to be operative also in isolated tissues exposed to high oxygen and the question arises of whether the lowering of oxygen consumption consequent upon such enzyme poisoning might not play a very significant rôle in the induction of those reactions seen in the tissue exposed to oxygen at high barometric pressure. In light of the possibility that the effects of oxygen poisoning might be attributed, in part at least, to the lowered oxygen utilization, a comparison of the effects of high oxygen on these tissues, with those effects produced by cyanide and by low oxygen, is of especial interest.

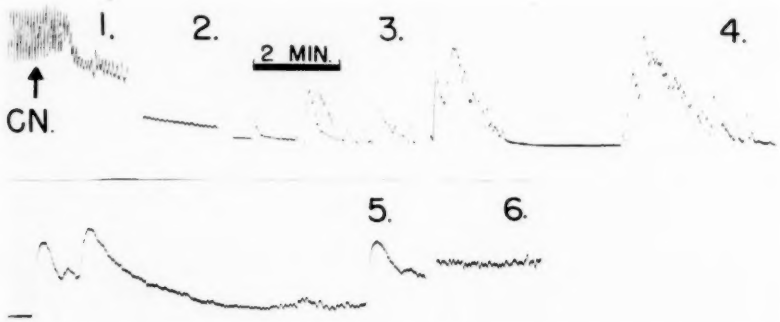

Fig. 4. The effects of sodium cyanide in high concentration on isolated longitudinal duodenal muscle of the rabbit: part 1, administration of cyanide (CN.) to give ultimate concentration of 1:5000 in Tyrode solution bubbled with oxygen at atmospheric pressure; part 2, 7 minutes after administration; part 3, 15 minutes after administration; part 4, 30 minutes after administration and beginning of compression of oxygen; part 5, after 50 minutes' exposure to oxygen at 75 pounds' pressure; part 6, 2 hours after decompression to atmospheric pressure.

To investigate the effects of cyanide on the smooth muscle under our experimental conditions, sodium cyanide was added to the oxygenated Tyrode bath in amounts sufficient to give an ultimate concentration of 1:1,000,000 or greater. Such procedures induced a fall in tonus and a decrease in frequency and amplitude of the rhythmical contractions of longitudinal duodenal tissue. In concentrations greater than 1:60,000 there occurred prolonged periods during which the rhythmical contractions were absent, interspersed by spasmodic waves of increased but unsustained tonus—effects which were essentially similar to those recorded in the more prolonged exposure to high oxygen pressure. The characteristic action of sodium cyanide in the higher concentrations on the longitudinal duodenal strip is shown in figure 4. The low tonus, the relative quiescence and irregularity of the rhythmic contractions interspersed by recurrent spas-

modic waves of increased tonus are features typical of the response of duodenal longitudinal muscle to oxygen at high pressure.

It is generally agreed that the action of cyanide on living tissue is due to the lowered oxygen utilization. Nevertheless a study of the effects of administering low percentages of oxygen on these tissues at atmospheric pressure is of interest here because unlike the cyanide effects, the responses induced by low oxygen administration are not dependent upon the poisoning of respiratory enzymes. A gaseous mixture of low oxygen (5 per cent) and nitrogen (95 per cent) previously warmed and saturated with water vapor was bubbled at atmospheric pressure into the Tyrode bath of a fresh strip of duodenal tissue. This gaseous mixture gave no significant alteration either in tonus or in the rhythmic contractions of the tissue. Gas mixtures of 3 per cent oxygen and 97 per cent nitrogen, however, induced a response which duplicated that induced by cyanide or high oxygen pressure. The administration of pure nitrogen brought about a more rapid onset of the same response and magnified the characteristic features. These results not only provide a comparison of low oxygen percentage effects with those of cyanide and high oxygen pressure but they also emphasize the wide margin of safety—so far as oxygen supply is concerned—within which the intestinal muscle *in vivo* functions under normal conditions.

Hyperoxic anoxia. The results presented thus far bring us to the belief that, while enzyme poisoning by high oxygen could conceivably lead by devious routes to a variety of changes in the tissue, all of which might contribute to the response of smooth muscle described above, the arresting similarity between the effects on isolated smooth muscle, of sodium cyanide, of low percentage of oxygen at atmospheric pressure and of oxygen at high barometric pressure is based on some underlying factor common to all three conditions and it appears this common factor is an anaerobic metabolism. In oxygen poisoning it would seem we have a paradoxical relationship in which a superabundant supply of oxygen gives rise to effects induced by oxygen want—a relationship which may perhaps be best described as a hyperoxic anoxia. In view of such probability it is not surprising that periodic breathing—a well known response to low oxygen administration—frequently occurs also in oxygen poisoning (Bean, 1932). Moreover, the finding of an increase in blood lactic acid in anesthetized animals breathing oxygen at high pressure lends further credence to the hyperoxic anoxia interpretation of acute oxygen poisoning.

In a series of seven of our present experiments the lactic acid content of the Tyrode bath exposed to oxygen at 75 pounds' pressure and in which the smooth muscle (duodenum) had shown some oxygen poisoning reaction, was determined. In six of these the lactic acid content was less than that found in the controls. This would perhaps seem to bring into ques-

tion the significance of the anaerobic metabolism as a factor in oxygen poisoning in these isolated smooth muscle preparations. It should be noted, however, that the oxidation of lactic acid intracellularly in intimate relationship with tissue fluids, or in the blood may be quite different from its oxidation in Tyrode solution under high oxygen pressure.

Even though it be granted that the parallelism in the responses of these isolated smooth muscle preparations to sodium cyanide and to oxygen at high pressure can be explained on the basis of a common anaerobic metabolism it must be recognized that there may be points of divergence in the toxic action of these two conditions. This is suggested by the finding that the effects of sodium cyanide and of high oxygen pressure administered in combination are not always directly and completely additive.

In order to determine whether there might be some unsuspected toxic substance released by the isolated tissue during its reaction to high oxygen pressure, the Tyrode bath was drawn off at the height of the muscle's response to high oxygen. This was then used as a bath for a normal piece of rhythmically contracting duodenum but the tonus and rhythm remained unaltered. If there is any toxic substance produced in the isolated smooth muscle during exposure to high oxygen, either it does not diffuse into the bath in significant amounts, or it is rapidly dissipated by high oxygen in the Tyrode solution or by decompression to atmospheric pressure.

SUMMARY

Exposure of isolated longitudinal duodenal muscle of the rabbit to oxygen at 75 pounds' pressure resulted in a progressive decrease in tonus, a decrease and irregularity in amplitude of the spontaneous rhythmic contractions and a decreased frequency of this rhythm to a point of periodic cessation interspersed by spasmodic unsustained increases in tonus. Decompression to atmospheric pressure reversed these effects.

Exposure of pyloric sphincter (rabbit) to high oxygen pressure likewise resulted in a decrease in tonus which was reversed by decompression.

The effects of high oxygen on intestinal smooth muscle in circulated animals are not necessarily dependent upon central nervous or hematogenous connections but may be explained as due to a peripheral influence of oxygen at high pressures.

Atropinization of duodenal smooth muscle preceding compression failed to alter the action of high oxygen pressure on this tissue. If the intrinsic nerve endings are affected by high oxygen it would appear that they do not mediate any effect via physiological sympathomimetic or parasympathomimetic substances.

The results indicate that the peripheral influence of oxygen at high pressure is due to a direct action on the effector cells themselves.

The finding that the effects of oxygen at high barometric pressure on isolated smooth muscle are so very similar to those effects induced not only by sodium cyanide but also by the administration of low percentages of oxygen at atmospheric pressure, is interpreted as indicative of a common causal factor operating in all three conditions.

Evidence is presented which supports the suggestion that this common factor is an increased intracellular acidity which in the case of the direct action of oxygen at high barometric pressure may arise from the diminished oxygen utilization consequent upon a poisoning of respiratory enzymes. Reasons are cited for believing that this interpretation remains valid despite the failure to demonstrate any increase in the lactic acid content of the Tyrode solution bathing the tissue exposed to high oxygen pressure.

The seemingly paradoxical relationship in acute oxygen poisoning where a superabundance of oxygen at high barometric pressure elicits responses typical of those induced by an oxygen deficiency and anaerobic metabolism is referred to as hyperoxic anoxia.

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OBSERVATIONS ON THE BEHAVIOR OF DIODRAST IN THE DOG[1]

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If a substance is completely removed from the blood on one renal passage, its blood clearance is a measure of renal blood flow (RBF); if it is completely removed from the plasma, while it is absent from or fixed in the blood cells during such passage, its plasma clearance is a measure of renal plasma flow (RPF). Regardless of the degree of completeness of renal extraction and regardless of distribution between cells and plasma, RBF is measured by the expression blood clearance/blood extraction, where blood extraction is

$$\frac{\text{arterial blood level} - \text{renal vein blood level}}{\text{arterial blood level}}.$$

If a substance is incompletely extracted from the plasma and is absent from or fixed in the cells, its plasma clearance/plasma extraction is a measure of RPF. If it is incompletely extracted from the plasma and some of it moves from cells into plasma during a renal passage, its plasma clearance/plasma extraction is higher than RPF. It is assumed that from the curve of momentary (observed) extractions can be plotted the mean extractions for the respective urine periods. The slight errors due to loss of water from blood into urine and to loss of materials through renal lymph are neglected.

Suitability of various substances for measuring RBF or RPF. Urea. Urea was used by Van Slyke, Rhoads, Hiller and Alving (5) as a reference substance for RBF determinations. It was later recognized that urea is not the most suitable substance, because of wide and transitory fluctuations in extraction (6). We confirm the following findings of Van Slyke, Hiller and Miller (7). First, that when urea is added to dog blood *in vitro* the equilibrium distribution between plasma and cells is attained very quickly (30 sec. of mixing, followed by centrifugation), with urea per 100

[1] Preliminary reports of this work have been made in the Proceedings of the Society for Experimental Biology and Medicine (1) (2) (3) and before the American Physiological Society (4). The work has been aided by a grant from the Commonwealth Fund.

cc. of water being the same in cells and plasma. Second, that urea diffusion *in vivo* is rapid enough that the cell/plasma urea ratio is the same in renal vein and in arterial blood. We have carried out the further experiment of making known dilutions of blood with urea free solutions and find that the equilibrium cell/plasma urea ratio is attained as quickly when urea is diffusing out from cells *in vitro* as when it is diffusing into them. It is thus confirmed that urea plasma clearance/urea plasma extraction will be greater than RPF, because of cell contribution of urea.

Creatinine. We confirm the following findings of Van Slyke, Hiller and Miller (7). First, that when creatinine is added to dog blood *in vitro* it enters the cells very slowly; the ratio of cell to plasma creatinine rises slowly and does not reach unity even after 5 hours. This is true with beef blood (slight rouleau formation with resultant high cell-plasma interface area) as well as with dog blood (marked rouleau formation). Second, it was confirmed that diffusion equilibrium of creatinine is attained *in vivo* at between 1 and 2 hours.

In order to find whether the rate of passage of creatinine from cells into plasma is the same as that from plasma into cells, the following experiment was performed. A dog was given 0.2 gram creatinine per kilo intravenously; 1 hour after the injection $\frac{[Cr]^w_c}{[Cr]^w_p}$ was found to be 0.99.[2] To 20 cc. of this blood were added 4 cc. of isotonic sodium chloride. A sample was taken from this and centrifuged immediately after mixing and another sample taken 20 minutes later. The ratio was raised immediately, due to the plasma dilution, and did not fall perceptibly in the next 20 minutes. The results are seen in table 1. This experiment shows that the passage of creatinine from cells into plasma as well as from plasma into cells *in vitro* is a very slow process.

Finally, we confirm Van Slyke, Hiller and Miller (7) in finding that the creatinine content of renal vein cells is the same as that of arterial cells, although renal vein plasma is of course lower than arterial plasma. It is thus confirmed that creatinine plasma clearance/creatinine plasma extraction is a measure of RPF.

Inulin. We confirm Van Slyke, Hiller and Miller (7) in finding that there is no inulin in the blood cells of dogs after a long period of *in vivo* equilibration. We also find the same on man. Inulin plasma clearance/inulin plasma extraction is thus a measure of RPF.

Diodrast. The organic iodine compound, diodrast (D), has been pro-

[2] It may be pointed out that a ratio of unity at a given time after a single intravenous injection does not necessarily mean that this ratio will be maintained thereafter, since, with a rapidly falling blood level, plasma creatinine falls faster than cell creatinine. The mere establishment of a unity ratio is, however, sufficient for the purposes of the experiment being described here.

posed by Smith and collaborators (8) (9) (10) as essentially meeting the conditions that it is completely extracted on passing active renal tissue and that it is absent from the cells.

Diodrast in cells. The statement of Smith (8) that D is absent from dog and human cells is based upon essentially complete recoveries from plasma of D added to drawn blood. We also find very little entrance of D into the cells of drawn blood, slightly more with dog than with human. Thus, D to the concentration of 4.55 mgm. iodine (I) per 100 cc. was added to freshly drawn heparinized human blood. After 4 minutes, with mixing, centrifugation was begun and carried on for 20 minutes at 2800 rpm. Plasma showed 7.32 and cells 0.53 mgm. I per 100 cc. Since plasma fraction of hematocrit reading (V_p) was 0.428, the amount of I found by analysis and hematocrit is 4.19 + 0.227 or 4.42 mgm. per 100 cc. of blood, a fair check. Assuming that 4 per cent of the volume of the packed cells

TABLE 1

Failure of creatinine to pass from cells into plasma in vitro in 23 minutes

V_p is plasma fraction from hematocrit. Calculated whole blood creatinine was obtained from plasma and cell analyses and hematocrit.

SAMPLE	TIME	V_p	PLASMA CREATININE	CELL CREATININE	WHOLE BLOOD CREATININE BY ANALYSIS	WHOLE BLOOD CREATININE, CALCULATED	CELL WATER	PLASMA WATER	$\frac{[Cr]^w_c}{[Cr]^w_p}$
			mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*gm. per 100 cc.*	*gm. per 100 cc.*	
3	10:25	60.2	18.6	13.8	16.8	16.6	70.66	94.44	0.99
4	10:28	66.5	13.9	13.2	13.9	13.7		95.83	1.29
5	10:48	66.7	13.8	13.2		13.7		95.83	1.29

is plasma, we find 0.25 mgm. I per 100 cc. of actual cells, about 3 per cent of the concentration in the plasma.

Diodrast to the concentration of 5 mgm. I per 100 cc. was added to heparinized dog blood and mixing accomplished by inverting every 3 seconds for 30 seconds. A sample of 5 cc. was removed at 30 seconds and its centrifugation for 20 minutes at 2800 rpm was begun 3 minutes after addition of D. The remainder of the blood stood undisturbed until 24 minutes after addition of D, when it was centrifugated. The sample obtained after 3 minutes showed 9.27 mgm. I per 100 cc. plasma and 0.88 mgm. per 100 cc. cells; assuming that 4 per cent of the packed cell volume is plasma we find 0.53 mgm. I per 100 cc. cells or 6 per cent of the plasma concentration. The sample after 24 minutes showed 8.75 mgm. I per 100 cc. plasma and 0.48 per 100 cc. cells; this gives a true cell content of 1.17 mgm. per 100 cc. or 13 per cent of the plasma concentration. Other similar experiments have given similar results.

The above findings show that D added to drawn blood passes from plasma into cells very slowly in human and somewhat more rapidly in dog blood. When, however, the equilibration is carried out *in vivo*, i.e., after and during intravenous injection of D, the rate of passage is much higher, equilibration being complete in 30 minutes or less. We give an intravenous injection of an amount of D and of inulin estimated to give the desired plasma concentration (priming infusion) and maintain this by a continuous or sustaining infusion; no sulphate or other diuretic is added. We have shown repeatedly that the plasma concentrations of creatinine, of inulin and of D are the same in simultaneous samples from an artery and from a systemic vein; the term "arterial" in this paper is applied to blood either from an artery or from a leg or jugular vein. The dogs were deprived of food but not of water since the evening before an experiment and were given 2.5 per cent body weight of water by stomach tube 45 to 60 minutes before the first urine period began. The sustaining infusion was begun 25 to 45 minutes before beginning the first urine period and immediately following the priming infusion. When blood levels were changing rapidly (after cessation of infusion) a time correction was made on the assumption that urine is formed two minutes before it enters the bladder. Urine was collected in graduate cylinders and the volume noted; the bladder was washed out with 20 cc. of saline from a syringe and the urine thus recovered noted from the increment in volume. Each complete urine sample was transferred to a 250 cc. volumetric flask and made up to volume. The excretion of the substances concerned in milligrams per minute was thus determined; from this and from the urine flow in cubic centimeters per minute the urinary concentration in mgm. per 100 cc. was calculated.

Not only the passage of D from plasma into cells but also that from cells into plasma is quite rapid, more so in the dog than in the human. This is seen when the blood level is allowed to fall; if the rate of fall is not too rapid the cell/plasma ratio remains constant. Whether the more rapid rate of passage *in vivo* means that the cell permeability is decreased *in vitro*, or that mechanical factors of the circulation are more effective in promoting equilibration than is mixing in the test tube, is not certain; perhaps both factors are operative. Furthermore, the equilibrium concentration of D in mgm. per 100 cc. of water is never as high in the cells as in the plasma. The significance of this is not clear but it apparently means that part of the water in the cell is either inaccessible to D or is incapable of dissolving D. These points are illustrated in table 2. Here the priming infusion was given rapidly (not through a catheter) and the sustaining infusion was at such a rate that the plasma D level was allowed to fall slowly for a while.

The average equilibrium value of the ratio $[D]_c^w/[D]_p^w$ for arterial blood is 0.58, with extremes 0.48 to 0.73. The average is of 33 observations

with plasma levels from 1.3 to 50 mgm. I per 100 cc.; the value of the ratio is independent of the plasma D level.

If the rate of fall of the blood D level is sufficiently high, as with cessation of infusion after a maintained high level, diffusion of D from cells

TABLE 2

Passage of D from cells into plasma in vivo to maintain constant cell/plasma ratio with slowly falling plasma level

Constancy of ratio is usually seen even where plasma level is falling much more rapidly than in this experiment. Priming infusion 12:20 to 12:22. Sustaining begun at 12:22 and continued throughout experiment.

TIME	ARTERIAL IODINE		$\frac{[D]^w_c}{[D]^w_p}$
	Plasma	Cells	
	mgm. per cent	*mgm. per cent*	
12:38	6.43	3.37	0.64
1:08	4.97	2.54	0.62
1:32	4.55	2.34	0.63
2:03	4.43	2.23	0.61
2:19	4.43	2.22	0.61

TABLE 3

Behavior of arterial and renal vein cell/plasma D ratios with rapidly falling plasma level

Priming infusion 10:34 to 10:44. Sustaining begun at 10:44 and stopped at 12:06. K1, 2/6/40.

TIME	ARTERIAL IODINE		ARTERIAL $\frac{[D]^w_c}{[D]^w_p}$	TIME	RENAL VEIN IODINE		RENAL VEIN $\frac{[D]^w_c}{[D]^w_p}$
	Plasma	Cells			Plasma	Cells	
	mgm. per cent	*mgm. per cent*			*mgm. per cent*	*mgm. per cent*	
11:03	29.4	11.35	0.47				
11:28	26.0	11.40	0.54	11:27	13.8	10.3	0.96
11:55	25.2	11.39	0.56	11:54	13.5	10.2	0.97
Infusion stopped at 12:06							
12:15	18.7	10.0	0.67	12:14	5.18	8.35	2.1
12:28	11.31	7.49	0.85	12:27	3.56	6.50	2.46
12:55	5.70	5.80	1.09	12:54	1.60	3.08	2.57
1:26	2.73	1.79	0.84				
1:35	2.31	1.46	0.81				

into plasma may fall behind, with a rise in the cell/plasma D ratio. Such a set of observations is recorded in the left half of table 3. Usually the arterial cell/plasma D ratio does not rise as high, even with a rapidly falling level, as in this experiment. Sometimes it does not rise at all and usually

does not exceed 0.80. This is in contrast with the findings on the human, where the rate of passage from cells into plasma is so low that $[D]^w_c/[D]^w_p$ may rise from an equilibrium value of 0.3 or 0.4 to 2.0, even though the rate of fall of plasma D from a given level, after cessation of sustaining infusion, is in general slower in man than in the dog. It is thus seen that both *in vitro* and *in vivo* the rate of passage of D from cells into plasma and vice versa is higher in the dog than in man, the rate in each species being much higher *in vivo* than *in vitro*. Details of the findings in man will be published elsewhere.

Contribution of D to urine by cells. Even though the cells contain a large proportion of D, one could still use D plasma clearance as a measure of RPF (if plasma D extraction were complete) provided no significant amount of D moved from cells into plasma during a renal passage. If, however, cell D moves into plasma on D depletion of the latter and becomes available for excretion, then urinary D per minute will be greater than original plasma contribution of D per minute and to this extent D plasma clearance will exceed RPF. That such a cell contribution of D does take place is indicated by the finding that renal vein cell D is always considerably lower than arterial cell D, where plasma D level is below that at which D clearance begins to be self-depressed (about 13 mgm. I per 100 cc.) and where diffusion equilibrium between arterial cells and plasma has been established. When the plasma D level is so high that extraction falls the difference between arterial and renal cell D naturally falls. The average ratio

$$\frac{\text{mgm. D per 100 cc. renal vein cells}}{\text{mgm. D per 100 cc. arterial cells}}$$

at equilibrium plasma levels below 13 mgm. is 0.77 (average of 11 observations), with extremes 0.64 to 0.90. However, renal vein cell D does not fall proportionally to renal vein plasma D; whereas, after equilibration at plasma D levels below that of self-depression of clearance, arterial plasma D is always higher than arterial cell D, renal vein plasma D is always lower than renal cell D. This merely means that while 30 to 16 minutes suffice for equilibration, the time of a renal passage does not suffice. An experiment which illustrates most of these points is shown in table 3.

We believe that the observed passage of D from cells into plasma has in large part taken place by the time the blood enters the renal vein rather than during the short time (average 2 to 3 min.) between the beginning of the drawing of the sample and the beginning of centrifugation. The evidence for this is the extremely low rate of passage in the *in vitro* experiments. The statement (2), that the finding that D plasma clearance/D plasma extraction is higher than independently measured RPF is "even more direct evidence of cell contribution of D than is the finding that

renal vein cell D is lower than arterial cell D," is not justified. The *in vitro* experiments, however, do indicate that there is a significant cell contribution of D to the urine.

Completeness of extraction of D from renal vein, blood or plasma. We find that renal extraction, $\frac{\text{A-RV}}{\text{A}}$, of D is never complete from either plasma or cells. When the arterial plasma level is below 13 mgm. I per 100 cc. the plasma extraction has averaged 0.74 (0.61 to 0.85), cell extraction 0.23 (0.10 to 0.36) and whole blood extraction 0.57 (0.46 to 0.66). Within this range of plasma levels (1.3 to 13 mgm. I per 100 cc.) there is but little correlation between plasma D level and completeness of extraction, while

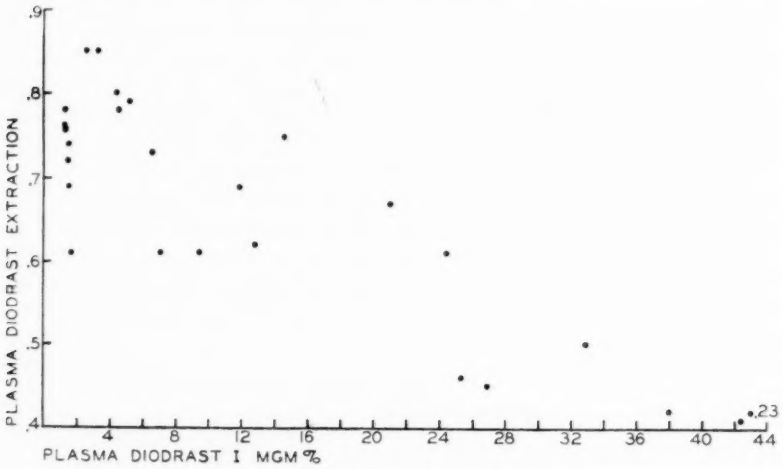

Fig. 1. Effect of varying plasma D level on plasma D extraction. Iodine (I) is a measure of diodrast (D). The anomalous extractions discussed in the text are not included in this figure. The point marked (0.23) designates an extraction of 0.23.

the extraction progressively falls at higher plasma levels. Some dogs consistently show a higher extraction than do others at a given plasma level. Figure 1 shows the relation between arterial plasma D level and plasma extraction; the data are from 4 dogs.

Anomalously low extractions. On one occasion out of a total of 44 renal vein bloods drawn and analyzed for D we have found renal vein plasma D higher than arterial plasma D; this undoubtedly means contamination by urine although none was seen entering the syringe; here the renal vein plasma inulin was also higher than the arterial. On two occasions we have found renal vein plasma D essentially the same as arterial. One explanation for this is that the supposed renal vein blood came from the renal artery. This is believed to be the correct explanation for one of

these cases, since the blood looked arterial. In the other case (K9, 3/28/40, R3), however, it seemed very probable that venous blood was obtained. If this is true, 3 possible explanations suggest themselves; first, that the puncture brings about a temporary suppression, presumably through nervous influences direct or reflex, of D excretion; second, that the needle point was close enough to the vena cava that caval blood to the exclusion of renal vein blood was obtained; third, that just the right amount of urine contamination occurred to bring the renal vein plasma D concentration up to the arterial. The second explanation seems inherently improbable. The third also seems inherently improbable; further light on this possibility may be afforded by the following consideration. If there had been a normal extraction with subsequent contamination by urine there would probably not have been time for the D from urine to come into diffusion equilibrium with the cells, since this would be an *in vitro* process. One would thus expect to find renal vein cell D lower than arterial cell D, as is always the case when a normal plasma extraction is observed. In the case under consideration, however, both plasma and cells of renal vein showed the same D content as arterial plasma and cells, which speaks against urine contamination while being compatible with all of the other possibilities.

In 5 other cases the extraction has been measurable but greatly below the lower limit of what we consider normal at the existing arterial plasma I level. In all 5 of these cases there had been difficulty in getting the renal vein sample. At arterial plasma I levels of 5.1, 7.9, 4.7, 7.9 and 12.6 mgm. the plasma extractions were 0.41, 0.32, 0.15, 0.33 and 0.22, respectively. In all of these cases the observed cell extraction also was very low, the ratio of cell extraction to plasma extraction being essentially the same as when plasma extraction is normal. As in the preceding paragraph, this speaks against contamination by urine as being responsible; we believe they represent partial temporary suppression of D excretion. It seems improbable that they could represent varying admixtures of renal vein and arterial blood, although this possibility is not excluded. The 8 anomalous D extractions discussed in this and the preceding paragraph are not included in figure 1 or in the preceding averages.

Whether such anomalous D extractions actually represent transitory suppression of D, whether this suppression is purely tubular and if so whether it is due to the action of inhibitory fibers to the tubular mechanism or merely to a transitory circulatory deficiency of the tubules cannot be answered without much further work. It is of interest in this connection to examine the simultaneous inulin extractions. If tubular excretion of D were completely suppressed while glomerular filtration was not completely suppressed the plasma extraction of D would be slightly less than that of inulin, slightly less because part of the plasma D is not filterable (11). If the suppression of tubular excretion resulted from activity of inhibitory

fibers to the tubular mechanism, with no circulatory change, inulin extraction should remain normal and should be slightly higher than D extraction. If, on the other hand, an adequate renal afferent vasoconstriction occurred, inulin extraction would fall. In the case (K9, 3/28/40, R3) where renal vein and arterial plasma D were essentially the same (extraction 0.04) the inulin extraction was correspondingly low (0.05). We interpret this as meaning that tubular excretion of D was completely suppressed and glomerular filtration greatly diminished, which suggests that in this case the effects were largely or wholly circulatory. This effect is very transitory, since both D and inulin plasma clearances for that 17 minute period were essentially the same as for another urine period in the same experiment in which D and inulin clearances and extractions were normal. In the 5 cases where D extraction was very low but higher than in the case just discussed, the inulin extraction was considerably lower than the D, indicating that tubular excretion of D had not been completely suppressed. Inulin extraction was itself considerably below normal, indicating partial suppression of glomerular filtration.

The question of transitory suppression of D excretion must be investigated by making urine collections at very short intervals while procedures designed to bring on such suppression are being carried out; in this way transitory falls in D output can be detected. We are not yet ready to report on this point. The question may be raised whether the renal vein puncture method of determining renal blood or plasma flows by observed extractions is satisfactorily reliable. Even though infrequent, the occasional finding of a very low extraction makes one wonder whether the extractions of the order of 0.6 or 0.7 may not represent milder degrees of the suppression process, if such exists. We cannot give a positive answer at this time. In a discussion at the 1940 meeting of the American Physiological Society Dr. Homer W. Smith stated that he and his collaborators had so far invariably observed anomalously low plasma D extractions on human subjects under various anesthetics, extractions so low that they obviously could not represent the mean extractions for the corresponding urine periods. Whatever the explanation may be, the difficulty seems much less likely to appear in the dog than in man.

Our present feeling is that in the dog by a judicious selection one can get D and inulin extractions which will yield a fair approximation to the mean extractions. Thus, if 5 out of 6 samples in one experiment show D extractions between 0.7 and 0.8 while the sixth, which may come between two normals, shows an extraction of 0.3, we feel justified in rejecting the low value and in concluding that the true mean extraction is between 0.7 and 0.8. Furthermore, the fact that D plasma clearance is independent of plasma D level up to a level of about 13 mgm. I per 100 cc., the same as that at which observed extraction begins to fall, indicates that our observed extractions, with the exceptions noted and discussed, are probably

representative of the mean extractions for the corresponding periods, i.e., that true mean extractions behave as do our observed extractions in being independent of plasma level up to about 13 mgm. I per 100 cc.

SUMMARY

The findings of Van Slyke and collaborators *a*, that urea comes into diffusion equilibrium between plasma and cells very rapidly *in vitro* and *in vivo*; *b*, that the *in vitro* equilibration of creatinine is very slow and the *in vivo* equilibration is slow enough that renal vein cell creatinine equals arterial cell creatinine, and *c*, that inulin is absent from blood cells *in vivo*, are confirmed.

The diffusion equilibration rates of D *in vitro* and *in vivo* are studied. The *in vitro* rate of passage of D between cells and plasma is very low. The *in vivo* rate of passage is high enough in the dog that renal vein cell D is lower than arterial cell D. It is concluded that there is a contribution of D to the urine by the cells in the dog.

At diffusion equilibrium the concentration of D per 100 cc. of cell water averages only 58 per cent of that in plasma water, this value being independent of the D level.

At arterial plasma D levels below that at which clearance begins to be self-depressed (about 13 mgm. I per 100 cc.) the extraction has averaged 0.74 (0.61 to 0.85) with but little correlation between completeness of extraction and plasma level between the levels 1.3 to 13 mgm. I per 100 cc. At higher plasma levels the extraction progressively falls. An anomalously low extraction is occasionally observed; possible explanations are considered.

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OBSERVATIONS ON INULIN AND DIODRAST CLEARANCES AND ON RENAL PLASMA FLOW IN NORMAL AND HYPOPHYSECTOMIZED DOGS[1]

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If plasma diodrast (D) extraction, $\frac{A\text{-}RV}{A}$, were complete and if no D moved from cells into plasma during a renal passage, D plasma clearance would be a measure of renal plasma flow, RPF. In practice, however, the two factors, incompleteness of D extraction and cell contribution of D (1) (2), work in opposing directions to disturb the relation between D plasma clearance and RPF, the first working to make the clearance lower, the second to make it higher than RPF. If the first factor alone were operative, D plasma clearance/D plasma extraction would be a measure of RPF; in so far as the second factor also is operative, this expression will be higher than RPF. If the cell contribution of D raises D plasma clearance less than the factor of incompleteness of D extraction lowers it, D plasma clearance will be less than RPF; the two factors might be balanced so that D plasma clearance would equal RPF. A comparison of D plasma clearances with simultaneous and independent RPF determinations in the dog is made in this paper. Observations on the relation between plasma D level and clearances and on the tubular excretion of D are also reported.

The general plan of the experiments has been described (2). The dogs were on a diet of Purina dog chow with a weekly supplement (given on Saturday) of 1 pound of raw meat. A previously described method for D (3) was used. Filtrate digestions were hand heated, urine digestions in a water bath. In its present published form the method yields slightly more consistent values on filtrates with hand heating than with the water bath; with urine either method of heating gives complete recoveries. We prefer the filtrates to unprecipitated plasma digestions. A determination of the percentage of D coming through the filtrates is made on each animal at 2 or 3 known levels, i.e., known amounts of D are added to blank blood,

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plasma and cells. These percentages continue to be remarkably constant. Inulin was determined essentially according to Corcoran and Page (4).

Relation of plasma D level to D plasma clearance. Since the extraction of D has been found independent of plasma D level between the limits 1.3 and 13 mgm. iodine (I) per 100 cc. (2), it follows that clearance should also be unaffected by changes in plasma level between these limits and this is found to be the case. Data on the relation between plasma clearance and plasma D level are recorded in figure 1. The higher the plasma level above 13 mgm., the less the clearance. The average at levels below 13 mgm. is 246 cc./minute/M² (26 observations on 6 dogs).

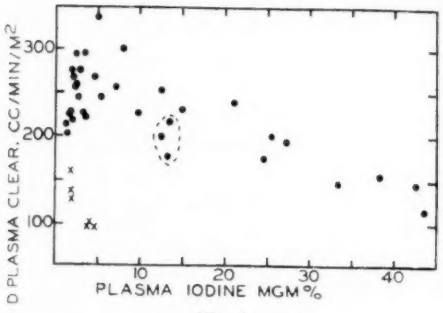

Fig. 1

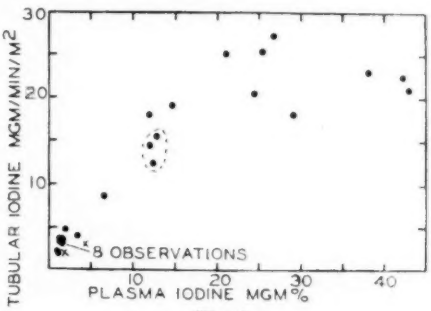

Fig. 2

Fig. 1. Diodrast plasma clearance as a function of plasma level. The clearance is essentially independent of plasma level up to about 13 mgm. I per 100 cc. and falls progressively with increasing levels above 13 mgm. The 3 points enclosed by a broken ring were obtained on a dog, K9, which had unusually small kidneys. The values designated by × are on 2 hypophysectomized dogs.

Fig. 2. Tubular output of D as a function of plasma level. The output is linearly proportional to plasma level up to about 13 mgm. I per 100 cc. and becomes independent of plasma level at about 20 mgm., i.e , diodrast Tm is attained, which has averaged 22.4 mgm. I/min./M². The enclosed points are from a dog, K9, which had unusually small kidneys. The values designated by × are on 2 hypophysectomized dogs; each represents the average of 3 observations.

Relation of plasma D level to tubular excretion of D. By means of simultaneous inulin clearances and calculations of the filterable fraction of D (5) one can determine the glomerular and thus the tubular excretion of D. The rate of tubular excretion, in mgm./min./M², rises linearly with rising plasma D level up to a level of about 13 mgm. I per 100 cc.; this is in agreement with the extraction and clearance findings. Above this level the slope falls and the curve becomes horizontal at 18 to 20 mgm. I per 100 cc., i.e., the maximal rate of tubular excretion of D (Tm) is attained. Data are reported in figure 2. Diodrast Tm averages 22.4 mgm./min./M², which is not much different from our figure of 25 mgm./min./M² on man (6).

Comparison of D plasma clearances with simultaneously determined RPF. Where V_p is the plasma fraction of the hematocrit reading (all bloods heparinized), RPF was determined by two methods simultaneously,

(a) $$\frac{\text{Inulin plasma clearance}}{\text{Inulin plasma extraction}},$$

(b) $$\frac{\text{D whole blood clearance} \times V_p}{\text{D whole blood extraction}}.$$

Whole blood D was determined from plasma and cell D analyses and hematocrit readings; this was occasionally checked by direct whole blood

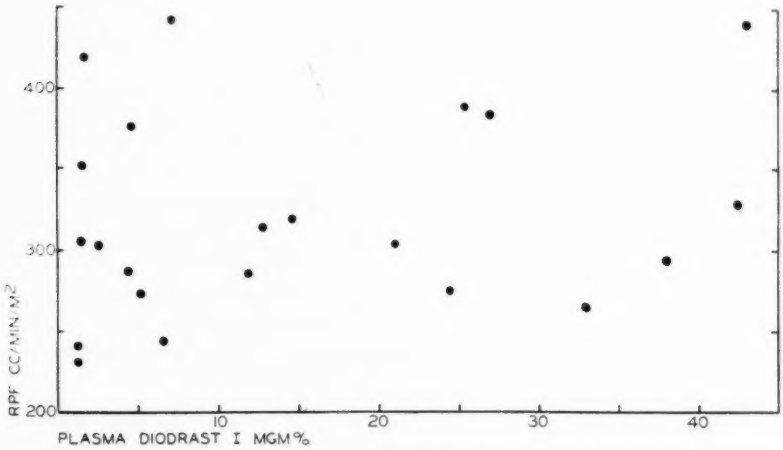

Fig. 3. True RPF values on 6 normal dogs, averaging 325 cc./min./M². As might be expected, these values are independent of plasma D level.

analyses. With observations on 22 periods, the average RPF values in cc./min./M² are, (a) 323 (215 to 436), (b) 326 (202 to 442). Data on RPF are shown in figure 3; the values are independent of the plasma D level. The recorded values are averages of the inulin and D values, which usually differ by less than 10 per cent. The D plasma clearance averaged 246 cc./min./M² when the plasma I level was below 13 mgm. per 100 cc., and the average D plasma clearance/D plasma extraction was 363 cc. (263 to 510). The latter average is higher than RPF (325 cc.) because of the cell contribution of D to the urine. Since 363 is 112 per cent of 325, it follows that for every 100 mgm. D contributed to the urine by the plasma, 12 mgm., on the average, are contributed by the cells. This is not a large but is a significant contribution. Evidence has already been advanced

(2) that this is a real contribution to the urine and not due merely to the passage of D from cells into plasma after the kidney has been passed.

The D plasma clearance is lower than RPF because the factor of incompleteness of extraction overbalances that of cell contribution. The average of all RPF values (22 periods), 325 cc., is 1.32 times the average of all D plasma clearances at plasma I levels below 13 mgm. (26 periods), 246 cc. However, of the 22 RPF periods only 13 were carried out at plasma I levels less than 13 mgm.[2] The average of these 13 RPF periods is 313 cc. and the average of the 13 simultaneous clearance values is 263 cc. Thus, in this series of 13 simultaneous determinations at plasma I levels less than 13 mgm., the average RPF is 1.19 times as great as clearance. This multiplier, however, has varied between 1.03 and 1.58, with no correlation between value of multiplier and plasma I level between the limits of 1.3 and 12.9 mgm. Variability of extraction is the factor chiefly responsible for variations in the multiplier.

The average renal whole blood flow is 655 cc./min./M^2, or 22.3 per cent of Marshall's (7) value of 2940 cc./min./M^2 for cardiac output in the dog. Corcoran and Page (8) found an average RPF of 250 cc./min./M^2 and RBF of 460 cc./min./M^2, using inulin and phenol red extractions. They used dogs from which the opposite kidney had been removed, with about 3 months allowed for hypertrophy of the explanted kidney. All of our dogs had both kidneys; the ratio of our values to those of Corcoran and Page is about what would be expected.

Filtration fraction. Inulin plasma clearance has averaged 91 cc./min./M^2. This is 28 per cent of average RPF; this filtration fraction has varied between the limits 0.18 and 0.40. The filtration fraction as indicated by the inulin extraction agrees well with the value obtained by inulin clearance/RPF, where RPF is D blood clearance $\times$ V_p/D blood extraction, i.e., the inulin and D findings are in agreement. In this series no experimental procedures designed to affect the filtration fraction have been carried out. The extreme value of 0.40 may be somewhat suspect, although it is difficult to see what technical difficulties could give erroneously high extractions; any accident to the renal vein blood should lower extraction. Our average of 0.28 is close to Corcoran and Page's average of 0.297 and slightly higher than Van Slyke, Hiller and Miller's (9) average of 0.223.

Table 1 presents data on a few periods; most of the points discussed are illustrated in this table.

Hypophysectomized dogs. In figures 1 and 2 are shown a few observations on D plasma clearance and tubular excretion in 2 hypophysectomized

[2] The remaining 13 of the 26 clearances at plasma I levels less than 13 mgm. were not accompanied by RPF determinations.

dogs. It is seen that both tubular output and plasma clearance are about half as high as in the normals. Inulin clearances (not shown in figures)

TABLE 1

Data of 5 periods on clearances and renal flows

A and RV refer to arterial and renal vein specimens, respectively. Uncorrected cell I means milligrams diodrast iodine per 100 cc. of centrifuged cells. Whole blood I is calculated from plasma and cell analyses and hematocrit readings. Mean D extraction is obtained from mean D levels for the respective urine periods, as determined by plotting analytical values against time. Glomerular D plasma clearance is obtained by multiplying inulin clearance by the filterable fraction of D as obtained from the constants of Smith and Smith (5); tubular clearance is total clearance minus glomerular clearance. Column (23) is to be compared with the average of columns (17) and (22), the excess representing cell contribution of D. Tubular I (24) is total I output minus glomerular I output as determined from inulin clearance and filterable fraction of D.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
DOG	URINE FLOW	PLASMA I		UNCORRECTED CELL I		WHOLE BLOOD I		MEAN D EXTRACTION		
		A	RV	A	RV	A	RV	Plasma	Cells	Blood
	cc./min./M²	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*			
K1	5.16	1.34	0.32	0.64	0.46	0.94	0.40	0.76	0.24	0.57
	5.31	1.32	0.32	0.64	0.46	0.93	0.40	0.76	0.24	0.57
K3	4.51	1.70	0.67	1.0	0.79	1.35	0.73	0.61	0.29	0.46
	1.56	1.55	0.49	0.91	0.56	1.25	0.52	0.69	0.36	0.58
	1.20	1.52	0.39	0.87	0.54	1.24	0.45	0.74	0.36	0.64

(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)
D PLASMA CLEARANCE			D BLOOD CLEARANCE	RBF (15)/(11)	RPF AS (16) × V_p	PLASMA INULIN		MEAN PLASMA INULIN EXTRACTION	INULIN PLASMA CLEARANCE	RPF AS (21)/(20)	(12)/(9)	TUBULAR I
Total	Glomerular	Tubular				A	RV					
cc./min./M²	*cc./min./M²*	*cc./min./M²*	*cc./min./M²*	*cc./min./M²*	*cc./min./M²*	*mgm. per cent*	*mgm. per cent*		*cc./min./M²*	*cc./min./M²*	*cc./min./M²*	*mgm./min./M²*
213	61.2	151.8	304	534	229	64.3	44.5	0.308	77.8	253	280	2.04
202	62.8	139.2	286	502	215	64.0	43.3	0.324	79.6	246	266	1.84
293	79	214	370	800	406	42.4	32.5	0.234	101	432	480	3.63
270	73	197	332	673	370	46	32.6	0.292	96.8	332	390	2.97
276	81	195	338	530	297	49.1	33.0	0.328	103	313	370	2.96

are also about half the normal. It thus follows that both glomerular and tubular outputs of D are about half the normal. We do not yet have observations on tubular outputs at high plasma D levels, Tm, on these

animals but this will probably also be low. The primary purpose in undertaking our inulin and D observations was to get further information on our earlier finding (10) that for several weeks after hypophysectomy or after the production of diabetes insipidus by appropriate hypothalamic lesions, the creatinine clearance was greatly reduced. The question arose as to whether this meant a low RPF or a low filtration fraction or both. We do not yet have observations on extractions or RPF (no renal explant hypophysectomized dogs), but the present finding that the ratio of inulin clearance to D plasma clearance is about normal indicates that the filtration fraction is normal and the low D plasma clearances probably mean a low RPF. Further observations on tubular function and on true RPF in these types of animals are in progress.

Discussion. It is seen that in the dog with one kidney explanted and the other present D plasma clearance is definitely less than RPF at plasma I levels between 1.3 and 13 mgm. per 100 cc. In the series where direct comparison was possible RPF averaged 1.19 times clearance. In general it may be said that if one multiplies the D plasma clearance (at plasma levels between the above limits) by 1.2 he will get a value which usually will differ from RPF by less than 10 per cent.

SUMMARY

Diodrast plasma clearance in dogs has averaged 246 cc./min./M² at plasma I levels between 1.3 and 13 mgm. per 100 cc.; the clearance falls progressively at higher levels.

Tubular output of D rises linearly with increasing plasma level up to about 13 mgm. I per 100 cc.; here the slope begins to fall and the curve becomes horizontal at about 20 mgm. Diodrast Tm, being attained at a plasma level of 20 mgm. I, averages 22.4 mgm. I/min./M².

True RPF is obtained, usually within 10 per cent, by multiplying D plasma clearance, obtained at plasma I levels between 1.3 and 13 mgm., by 1.2. Renal plasma flow has averaged 325, renal blood flow 655 cc./min./M².

About 11 per cent of the D entering the urine comes from the blood cells.

The filtration fraction of plasma has averaged 0.28.

The inulin and D plasma clearances and the rate of tubular output of D in the hypophysectomized dog are about half the normal. This indicates a low RPF with an essentially normal filtration fraction.

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FREQUENT FAILURE OF A SINGLE INSEMINATION TO ACTIVATE THE CORPORA LUTEA OF THE RAT SUFFICIENTLY FOR IMPLANTATION OF FERTILIZED OVA[1]

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In a previous communication (Ball, 1934) it was shown that pseudopregnancy in the intact rat followed sterile mating invariably only if two or more vaginal plugs had been deposited. In about one-third of the cases in which mating was terminated after the formation of the first plug estrous smears recurred within 4 or 5 days as if no mating had taken place. In the light of this fact it seemed probable that a similar failure of the pseudopregnancy mechanism might prevent the implantation of healthy, fertilized eggs after mating with fertile males if only one plug were formed. A series of one-plug matings was therefore undertaken in order to discover if such a failure does in fact occur when the ova are fertilized as well as when they are not.

EXPERIMENTAL PROCEDURE. Seventy-three female rats of a mixed hooded strain were used for these matings. They were between 3 and 4 months old at the beginning of the experiment. Most of them had borne one litter but the litters had been discarded and the mothers were started on the experimental breeding after a rest of not less than 10 days. They were fed Purina Dog Chow and lettuce with additional dried whole milk during pregnancy. They were, of course, kept segregated except during the observed matings.

When vaginal smears indicated probable sexual receptivity each female was allowed to copulate with a male of proved fertility until one plug had been formed. She was then returned to her home cage. Vaginal smears were taken daily after this until the appearance of either the red blood cells indicative of pregnancy or cornified cells indicating a new estrus and failure of gestation (Evans and Burr). In the former case smears were discontinued and the animal was set aside as pregnant. If, however, an estrous smear appeared first she was mated again, usually to the same male, and this time three plugs were permitted before the animals were sepa-

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rated. In about half of these three-plug matings a vasectomized male was substituted for the fertile male after the first plug had been deposited in order to show that the improved reproductive performance after three-plug matings over that resulting from one-plug matings was not due to the presence of a greater concentration of spermatozoa in the female sex tract. Since the results were the same whether fertile or sterilized males were used for the second and third plugs all cases have been tabulated together for the following discussion.

After the three-plug mating the female was treated as she had been after the one-plug mating. If she again failed to become pregnant she was considered sterile and her one-plug record was discarded although occasionally such a female would produce a normal litter if mated again.

The litters born after these matings were discarded at birth and the same procedure was repeated once more after a rest period of 10 days or more.

Many of the single plugs were examined microscopically and in every case active spermatozoa were found in normal profusion.

A second group consisting of 19 rats of an albino strain were treated in exactly the same way. These animals are reported as a second group only because their performance was somewhat different. They were the same age as the hooded rats, were bred during the same months and were kept in the same cages. They were not considered a separate group until it was discovered that all but one had become pregnant after one-plug matings, a much higher fertility than that of the hooded animals.

Results. In the first group, the hooded animals, there were 109 one-plug matings in which the fertility of both male and female was proved by production of a litter as a result of either that mating or the subsequent three-plug mating. Of these 109 one-plug matings, 71 (65 per cent) resulted in litters while 34 (31 per cent) were followed by estrus in 4 or 5 days as if no mating had occurred, showing that in about one-third of the cases one plug is not enough to stimulate the corpora lutea sufficiently to permit implantation. In only 4 cases (4 per cent) did these one-plug matings fail to produce litters for reasons other than failure of the mechanism which produces pseudopregnancy in sterile mating. In each of these 4 cases (1 resorption and 3 pseudopregnancies) the reproductive history of the animal offered some other evidence, such as very small litters or too long non-copulatory cycles, that her reproductive system was not quite up to normal.

In no case did a 4-day or 5-day cycle follow a three-plug mating. If pregnancy failed, the animal was always at least pseudopregnant. In other words, three plugs were always an adequate stimulus to produce functional corpora lutea, although one plug was often not. The outcome of these matings was, therefore, almost precisely what had been expected in the light of the previous experience in mating hooded rats with vasec-

tomized males, even the two to one proportion of success and failure remaining the same for the hooded animals.

The albinos, however, were apparently more reactive to the stimulus of copulation for in this group 31 (94 per cent) of the 34 one-plug matings resulted in litters and only 2 (6 per cent) of them (both in the same female) were followed by short cycles. There was one pseudopregnancy.

The albino rats had been raised in another laboratory and 27 of the hooded animals had come from a third laboratory so that it cannot be said with certainty that the difference in performance was due to strain difference but this seems the most probable explanation since all animals appeared to be in good health at the time of the experiment. A difference between these hooded rats and another albino strain in responsiveness to injected hormones has already been noted (Ball, 1939).

Although there would seem to be no reason to doubt that the eggs were fertilized by a single plug as well as by three since one fertile plug was as successful as three in producing young if it was followed by infertile plugs, nevertheless several females were examined when they showed estrous smears four days after mating. To obtain the eggs one uterus was carefully tied off as close to the cervix as possible and then removed with its contents and with the ovary attached. The other uterus and ovary were left intact in order to test fertility as usual by three-plug insemination. The contents of the removed uterus and tube were examined by Dr. Carl G. Hartman to determine the presence and condition of eggs.

A number of the animals subjected to this examination proved to be sterile when tested later by three-plug matings. These cases, like the other cases of sterile animals, were of course discarded since it was the purpose of this experiment to determine the relative number of successful pregnancies after one-plug and three-plug matings. In order to do this it was obviously necessary to use only animals that were capable of producing litters, given adequate stimulus. It is, however, of interest to note that the eggs found in the sterile animals were uniformly unfertilized and disintegrating.

In contrast to this defectiveness of the gametes in the animals discarded as sterile, the eggs found in the four fertile females examined were fertilized and developing normally showing that it was not the zygotes but the maternal endocrine system which was responsible for the failure of pregnancy in these cases.

It is apparent from these studies that for some rats a single insemination is insufficient stimulus to insure inception of the chain of events necessary to prepare the uterus for implantation and that this process is quite independent of the fertilization and early growth of eggs. Failure of pregnancy may be due to either or both. If only gametes fail, pseudopregnancy results. If only preparation of the uterus fails, due to too little cervical

stimulation, the next estrus is not inhibited and normally developing eggs are contained in a proestrous uterus. If both fail there is a short cycle but the proestrous uterus contains only disintegrating eggs. Deposition of three plugs prevents the occurrence of the second and third of these conditions. It may be added that rats usually make three or more plugs if they are simply left together over night when the female is in heat. But occasionally only one plug will be made due to low sexual excitability of one or both partners.

SUMMARY

Approximately one-third of 109 single-insemination matings of fertile rats were followed by estrous smears within 4 or 5 days as if no mating had taken place. Normally developing fertilized eggs were found in the uterus of four such animals examined at the time of proestrous smears four days after mating.

This frequent failure of pregnancy after deposition of a single vaginal plug is considered to be due to the same failure of the pseudopregnancy mechanism that had occurred in a comparable proportion of cases when similar matings had been made with vasectomized males. Since short cycles never occurred after the deposition of three plugs by either fertile or sterile males it is concluded that for the animals used in these experiments, a mixed hooded strain, one plug is insufficient stimulus to insure inception of the chain of events necessary to prepare the uterus for implantation whether there are eggs to implant or not.

A small group of rats of an albino strain responded to the stimulus of one plug much more readily than the hooded animals.

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THE REACTIONS OF PARTIALLY DENERVATED SMOOTH MUSCLE TO ADRENALINE AND SYMPATHIN

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Although it is generally agreed that skeletal muscle is composed of motor units which may contract independently of one another, it is still doubtful whether there are similar units in smooth muscle. Since the bulk of the histological evidence favors a sparseness of innervation resulting in one nerve fiber to more than one smooth muscle cell (for references see Cannon and Rosenblueth, 1937), the existence of units composed of single smooth muscle cells is improbable.

Denervation of the nictitating membrane results in an increased sensitivity to adrenaline and to sympathin (see Cannon and Rosenblueth, 1937). This hypersensitivity decreases and disappears as regeneration of the previously severed postganglionic fibers progresses (Simeone, 1937).

Lawrentjew and Borowskaja (1936) have shown histologically that section of some of the postganglionic fibers distributed to a smooth muscle effector results in a partial denervation. It seemed plausible that partial denervation of the nictitating membrane might sensitize only some cell groups. Such a result would support the concept of a relative unitary organization of smooth muscle. The present study was undertaken to answer this question.

METHOD. Cats were used. One superior cervical ganglion was exposed aseptically under ether anesthesia and some of the postganglionic fibers were severed. For control observations the ganglion of the opposite side was either removed, preganglionically denervated, or left intact. Seven to 14 days were allowed for sensitization to develop. The cats were then used under dial anesthesia (Ciba, 0.8 cc. per kgm. intraperitoneally).

Isotonic contractions were recorded from one or both nictitating membranes by levers providing a 20-fold magnification and a load of 3.5 grams. Injections of adrenaline (adrenalin, Parke, Davis and Co.) were made into the femoral vein in dilutions of 1:50,000 to 1:200,000 in 0.9 per cent NaCl. One dilution was employed in any series of observations. A constant time (10 sec.) was used for each injection. For nerve stimulation shielded electrodes were placed on either the pre- or postganglionic fibers of the

cervical sympathetic. Maximal shocks were delivered at various frequencies from a multivibrator circuit discharging through a transformer. Each frequency was applied for a constant time (20 sec.).

RESULTS. A. *Responses to adrenaline.* Series of responses of partially denervated nictitating membranes to varied doses of adrenaline were recorded simultaneously with the responses of control membranes, normal or with total pre- or postganglionic denervation. Adrenaline was given in either gradually increasing or gradually decreasing amounts. Random doses did not yield consistent results. This inconsistency is probably due to changes in the sensitivity of the muscle cells during the course of the

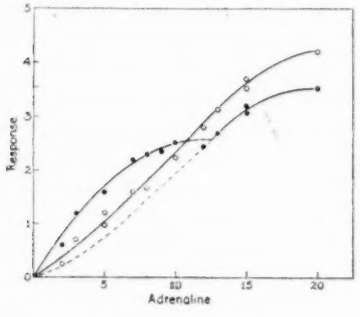

Fig. 1

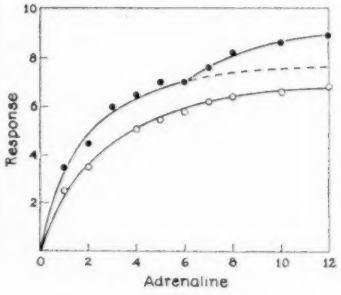

Fig. 2

Fig. 1. Dose-response curves for adrenaline. Ordinates: maximal height of responses. Abscissae: adrenaline (γ). Dots: partially denervated nictitating membrane; the broken line is the inferred extrapolation of the upper segment of the curve. Circles: normal control membrane of the opposite side.

Fig. 2. Dose-response curves for adrenaline. Ordinates and abscissae as in figure 1. Dots: partially denervated membrane; the broken line is the inferred extrapolation of the lower segment of the curve. Circles: totally denervated control membrane of the opposite side.

experiment, for injections of adrenaline result in a gradual desensitization of innervated muscles (Simeone, 1938).

The control membranes yielded smooth, continuous curves when the maximal height of response was plotted against the corresponding dose of adrenaline (Rosenblueth, 1932; figs. 1 and 2). The curves from the partially denervated muscles, on the other hand, invariably showed a discontinuity or break (figs. 1 and 2). The level in the curve at which this break occurred varied from one animal to another, probably because the fraction of postganglionic fibers interrupted by the previous section was also variable (see below).

An examination of figures 1 and 2 reveals that the partially denervated membrane responds to relatively large doses of adrenaline like the normal

control (upper segment of the broken curve, fig. 1), whereas it reacts to small doses like the totally denervated control (lower segment, fig. 2). The breaks can be readily interpreted if it is assumed that partial denervation leads to sensitization of a fraction of the total number of cells in the muscle. The lower segment of the curves would then be due almost exclusively to a contraction of the sensitized elements. Large doses of adrenaline would, on the other hand, activate not only the sensitive but also the normal elements.

B. *Responses to preganglionic nerve stimulation.* Series of responses were obtained with various frequencies of nerve stimulation at constant intensity. The magnitude of the response was measured at the end of 20 sec. The results of a typical experiment are plotted in figure 3. As in the case of the curves plotted from the response to adrenaline, the present

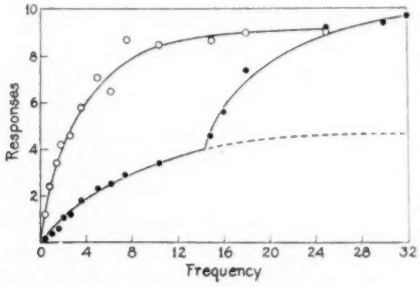

Fig. 3. Frequency-response curves. Ordinates: height of responses after 15 sec. of stimulation. Abscissae: frequency of maximal stimulation of the preganglionic sympathetic fibers. Dots: partially denervated membrane. Circles: normal control membrane.

curves were not smooth but showed a break. The position of this break varied from animal to animal, depending probably on the number of postganglionic fibers cut. In any given animal the break occurred at the same height of response in successive curves built by successive series of observations, but not at the same frequency. This shift of the critical frequency may be explained as due to a progressive exhaustion of the sympathin liberated and to a relative desensitization of the denervated cells (see Simeone, 1938).

The curves in figure 3 differ from the smooth curves obtained by Rosenblueth and Rioch (1933) in acute partially denervated membranes. The difference may be attributed to the increased sensitivity to sympathin which chronic denervation produces in some cells. Thus it is probable that the initial portion of the broken curve in figure 3 represents the response mainly of innervated cell units, while the final portion represents the addi-

tion of denervated (i.e., sensitized) cell units activated by the diffusing mediator. The position of the break in the curve may accordingly be taken as an indicator of the critical concentration of mediator at which a significant diffusion begins.

C. *Postganglionic nerve stimulation.* In 3 cats response-frequency curves were plotted by stimulating first the preganglionic and later the postganglionic fibers. The results were similar for both types of stimulation—i.e., the curves showed a break at approximately the same height of response. It may be inferred that the breaks are to be attributed to some condition in the effector, not in the ganglion.

D. *Effects of removal of the eyeballs.* Brücke (1938) reported that the increased sensitivity to adrenaline of the preganglionically denervated membrane disappears after removal of the eyeball. He inferred that the smooth muscle of the membrane is not sensitized by such denervation, and that the increased response obtained with the eyeballs intact is due exclusively to a greater reactivity than normal of the smooth muscle which protrudes the eye.

Rosenblueth and Cannon (1939) were unable to confirm Brücke's report. In the present study 2 cats were tested as follows. With the eyeballs intact as usual a response-frequency curve for preganglionic nerve stimulation and a response-dose curve for adrenaline were constructed. The eyeballs were then excised and the observations were repeated. The results were uninfluenced by the removal of the eyeballs.

E. *Comparison of the adrenaline and of the nerve stimulation curves.* In the broken curves obtained with adrenaline (fig. 1) the lower segment was attributed to the sensitized and the upper segment to the normal cells. Conversely, in the curves plotted from responses to nerve stimulation, the lower segment was interpreted as due to the contraction of the innervated elements, and the upper segment to the addition of the denervated elements. If these inferences are correct it follows that when only a small fraction of the nerve fibers are cut, the break in the curves should be at a relatively low level of response for adrenaline and at a relatively high point for nerve stimulation. The converse should be true if a large fraction of the nerve fibers are interrupted. These corollaries were supported by the experiments. Whenever the break in the adrenaline curve was low, that in the frequency curve was high and *vice versa.*

Discussion. The results (figs. 1, 2 and 3) indicate that degeneration of some of the postganglionic fibers which supply the nictitating membrane cause a relatively sharp division in the muscle. Some cells retain their normal sensitivity to sympathin and adrenaline while others are more sensitive. Such differentiation is absent in normal or totally decentralized or denervated membranes.

Rosenblueth and Rioch (1933) showed that stimulation of a fraction of

the total nerve supply of a normal membrane tends to activate all the muscle cells if the frequency is sufficiently high. Rosenblueth and Morison (1934) showed that diffusion of sympathin into the blood stream occurred only when the stimulating frequency reached a critical value (3 to 12 per sec.). The present observations agree with these reports. When the frequency of stimulation attains a critical value (4 to 14 per sec.), a diffusion of sympathin takes place from the innervated into the denervated cells. The difference between the present data and those of Rosenblueth and Rioch (1933) lies in the fact that the diffusing sympathin reaches elements whose sensitivity is increased by previous denervation.

If the suppression of a continuous tonic delivery of sympathin is the factor responsible for sensitization after denervation, it may be inferred that the tonic discharge of nerve impulses occurs normally at relatively low frequencies, insufficient for significant diffusion into neighboring elements, since only some cells are sensitized when the denervation is partial.

The bearing of the present data on the problem of the existence or lack of existence of motor units in smooth muscle is complex. Since some elements in the muscle are differentially affected by partial denervation, it is apparent that a relative division into units is defensible. Such a division is obviously in disagreement with Bozler's (1939) recent suggestion that smooth muscles are a single unit of syncytial structure, like the heart. On the other hand, since with sufficiently high frequencies of stimulation the whole muscle may be affected by only some of the nerve fibers it is also obvious that there is no unitary arrangement similar to that of skeletal muscle, as Eccles and Magladery (1939) have suggested.

The most tenable view at present is that the smooth muscle of the nictitating membrane is neither like the heart nor like striated muscle, but occupies an intermediate position. The number of smooth muscle cells controlled by a nerve fiber is variable, increasing when the frequency of the nerve impulses increases (Rosenblueth and Rioch, 1933). With low, physiological frequencies the sphere of action of a nerve fiber would then be relatively small. Section of some nerve fibers does not modify this low frequency. Increased sensitivity becomes apparent in only some muscle cells.

SUMMARY

Chronic partial postganglionic denervation of the nictitating membrane divides the smooth muscle into groups of cells which react in a quantitatively different manner to adrenaline and nerve stimulation.

This difference is due to changes in the effector and not in the superior cervical ganglion.

This action is independent of the presence of the eyeballs.

An explanation of the results is given which entails a clearer definition of the smooth muscle units of the nictitating membrane.

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EXCITABILITY OF THE EXCISED AND CIRCULATED FROG'S SCIATIC NERVE

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The passage of an electrical current through a nerve places it in a state of altered excitability which was called electrotonus by the discoverer of the phenomenon, DuBois Reymond (1849). Pflüger (1859) studied the effects of constant current on nerve with extreme care and found that during the passage of the current there was a lowering of the threshold to induction test stimuli at and near the cathode, while the threshold to such stimuli was raised at and near the anode. The magnitude of the effect was found to be a function of the proximity of the point tested to the constant current electrode and of the strength of the applied current. When the constant current was interrupted the effects at both anode and cathode were reversed. Pflüger concluded that the electrotonic changes of excitability were the essential phenomena of excitation. Some recent workers, Bishop and Erlanger (1926), Bishop (1928a, b), Erlanger and Blair (1932), Bogue and Rosenberg (1934), Rosenberg (1937), associate the excitation phenomena with the development of electrotonus at the cathode. Hill (1936) and his co-workers have, however, postulated the existence of excitatory processes which are independent of electrotonic phenomena.

As experimental data have accumulated, theories of excitation have been developed in an attempt to coördinate the data. Among them may be mentioned the theories of DuBois Reymond (1849), Nernst (1908a, b), Lapicque (1926), Blair (1932), Rashevsky (1933), Monnier (1934) and Hill (1935, 1936). All the earlier ones have proven inadequate since they apply only to particular cases. The theories of Hill, Monnier and Rashevsky attempt to treat mathematically the general phenomena of excitation in terms of two processes. At the cathode one process is rapid, tending to excitation, and the other is slower, giving a recovery, inhibition, adaptation or accommodation, while opposite effects are produced at the anode. Offner (1937) and Young (1937) found that where the intensity-time relations for any type of stimulating current were concerned the theories of Rashevsky and Hill were identical. Rushton (1937)

showed that the same second order differential equation expressed the assumptions of both Hill and Monnier. When extended to include the formulations of Rashevsky and Young (1937), the equation becomes

$$\frac{d^2\theta}{dt^2} + (k_{11} + k_{22})\frac{d\theta}{dt} + (k_{11}k_{22} - k_{12}k_{21})\theta$$

$$= (B - A)\frac{dI}{dt} + [A(k_{21} + k_{22}) - B(k_{11} + k_{12})]I, \quad (1)$$

where θ is the difference between the excitability at any time and that at rest, $\theta = (U - V) - (U_o - V_o)$ as given by Hill, k_{11}, k_{12}, k_{21} and k_{22} as given by Young are velocity constants, and I is the applied current. From Hill, $k_{11} = -1/k$; $k_{22} = -1/\lambda$; $k_{12} = O$; $k_{21} = 1/\beta$; $A = C$; B = O, and similar identifications are found from Rashevsky and Monnier. It is obvious by inspection that the term in I is necessary for an electrotonus to be established after a sufficient length of time, but this term also requires an excitation by sufficiently large currents—no matter how slowly they rise (cf. Cole, 1933).

All the authors have treated the minimal current gradient by making the coefficient of I zero and excluding electrotonus from the formulation. These theories require that the threshold be independent of any constant direct current and that the alternating current threshold approach infinity as the frequency approaches zero, while the threshold for anode break must be the same as for cathode make. According to the theories a break response must occur after the current has flown for some time, 200 msec. or longer. The absence of a break response cannot be explained by the recent theories.

An extensive group of experiments performed on the circulated frog's sciatic-gastrocnemius showed that it was nearly impossible to secure break responses after constant currents had flowed for intervals of one quarter to five seconds. Break responses were obtained only with currents of injurious strength or after the circulation had been impaired. In the same preparations the make responses were usually tetanic even with stimuli of threshold strength. These responses showed that the constant current was effective as a stimulus over relatively long time intervals. Such results suggested the study of the action potentials of the nerve itself under similar conditions. The cathode ray study showed that the nerve was responding repetitively to the constant current. These divergences from the theoretical predictions and the frequently recorded responses of excised nerve (Bishop and Erlanger, 1926; Bishop, 1928b; Erlanger and Blair, 1932), indicated the desirability of studying the adequately circulated sciatic nerve when subjected to the passage of constant current.

MATERIALS AND METHODS. The test shock method of Bishop (1928b) and Erlanger and Blair (1932) was used to measure the excitability of the alpha fibers of the frog's sciatic nerve during and after the passage of direct current. The Pickerel frog was used exclusively. All frogs were equilibrated to temperatures between 20° and 23°C. for at least one week before experimentation and all data were obtained at the same temperatures. For stimulation and recording the action potential the nerve was either exposed in the animal, or excised and placed in a moist chamber as desired. In the circulated animals, spinal section was performed under ether anesthesia two or more days before use. Hemorrhage was kept minimal.

The constant currents (two megohms in series with the stimulating electrodes) and test shocks were applied through the same pair of electrodes spaced one centimeter apart. A relay that opened a series of contacts tripped the cathode ray sweep and applied both stimuli. A suitable micrometer adjustment permitted the application of the test stimulus at any time from 2 msec. before to 15 msec. after the make of the constant current. Silver-silver chloride electrodes were used for stimulation. An accessory circuit, which included a galvanometer that could be short-circuited, permitted balancing any polarization potential at the stimulating electrodes. After conduction through about 3 cm. of nerve the action potentials were led to a resistance-capacity coupled amplifier, provided with a differential input (Toennies, 1938), which amplified them for observation and measurement on the oscillograph.

The half maximal alpha spike (Erlanger and Gasser, 1924, 1937), served as the index of nerve activity. The test voltage required to evoke the half maximal response was recorded and the rheobasic constant current determined. Then the constant current was adjusted to the desired subrheobasic value and applied together with a test shock at the desired time in relation to the constant current make. The test voltage was now adjusted to just produce a half maximal response in the presence of the constant current. The required test voltage was expressed as percentage of the test voltage necessary to evoke a half maximal response when applied alone.

EXPERIMENTAL RESULTS. *Excised nerves.* Nerves that were removed from the animal and immediately placed in a moist chamber showed clearly the inability of the constant current to maintain a constant level of altered excitability with increasing durations of current flow. The adjustment of the nerve to the constant current was, however, very seldom complete; that is, the excitability remained at a level different from the resting condition throughout the time of current passage. Figure 1 shows clearly the magnitude and time course of these excitability changes at both electrodes as functions of the applied constant current strength. It

should be recalled that at the cathode the excitability is increased while at the anode it is decreased. The maximum effect of the subrheobasic constant current is attained between 0.5 and 1 msec. after its make and the effect begins to decline after about 2 msec. of current flow. This decay of the electrotonic state may be considered as an inhibition, adaptation, or accommodation.

In order to establish a method of measuring these variations of the electrotonic state it is necessary to consider briefly the data from circulated nerves (fig. 3). When, in the circulated nerve, the strength of the test

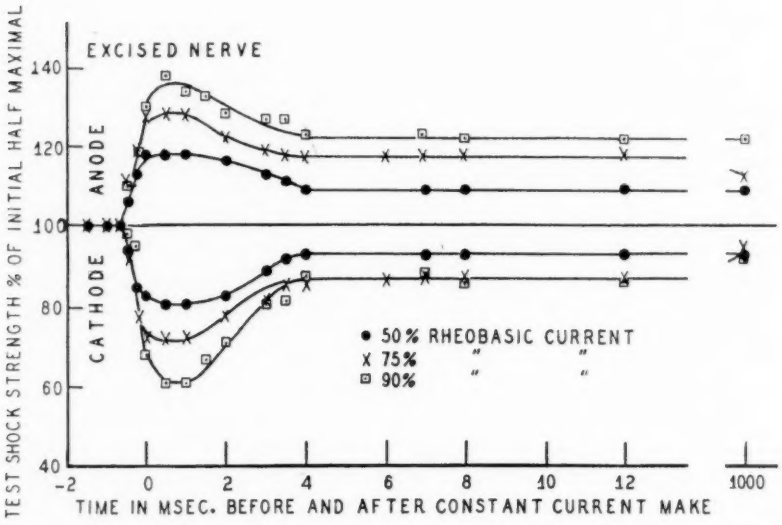

Fig. 1. Excised nerve. Time course of excitability at anode and cathode of subrheobasic constant currents, as measured by the test shock method.

Abscissae: Time of application of test shock in relation to constant current make.

Ordinates: Test shock strength as percentage of the test shock which, when applied alone, evokes a half maximal alpha action potential.

shocks required to evoke a half maximal alpha spike, in the presence of constant currents, is plotted against the strength of the constant currents, expressed in rheobases, the points fall on a straight line (fig. 2, circulated nerve). This line represents the summation of the test shock and constant current and it passes through the origin where the test shock is just strong enough to evoke a half maximal alpha spike with zero constant current and through the point on the axis of constant currents where this is just strong enough to evoke a half maximal response with the test shock zero (fig. 2, circulated nerve). It is seen, therefore, that in the circulated

nerve over a range of constant currents from 50 to 100 per cent of rheobasic the test shock increment is directly proportional to the constant current decrement or that the converse is true. It is also evident (fig. 2, circulated nerve) that in the circulated nerve regardless of the duration of

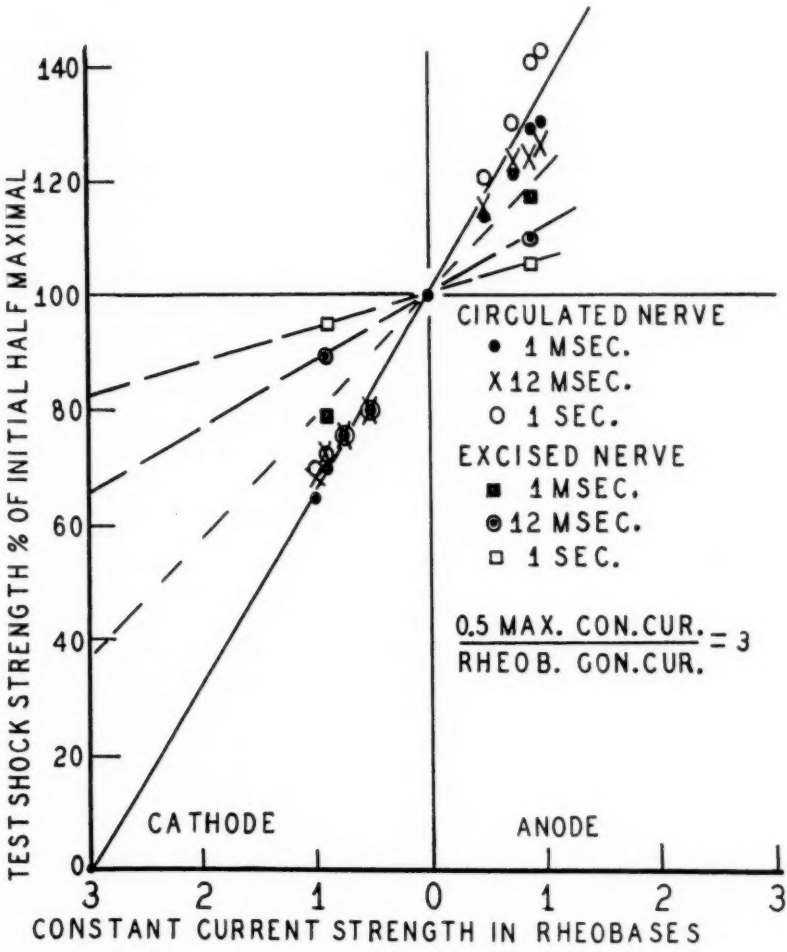

Fig. 2. Comparison of excised and circulated nerve.

Abscissae: Constant current strength at anode and cathode in rheobases.

Ordinates: Test shock strength. For the excised nerve, the point of intersection of the line through the test shock strength and the abscissa of ½ maximal constant current strength is a measure of the magnitude of the "decay of electrotonus."

1, 12, etc. is the time of application of the test shock after constant current make.

the constant current, up to one second, the points fall on the same straight line. There is, therefore, no evidence of a decay of the electrotonic state from its maximal value during the time intervals studied. The test shock is then a valid measure of the state of the electrotonus produced by constant currents of strengths varying from 50 to 100 per cent of rheobasic and of durations up to one second. Under conditions such that the electrotonic state rises and falls, the fall will be indicated by a rotation, toward the axis of 100 per cent test shock strength, of the line through the point having as its coördinates, test shock strength, and constant current strength used (fig. 2). When there is no rotation, that is when the experimental points fall on the line passing through the origin (100 per cent test shock strength, 0 constant current) and the half maximal constant current intercept, the decay of electrotonus, inhibition, adaptation or accommodation will be zero. When there is complete rotation, that is, to the axis of 100 per cent test shock strength, the decay of electrotonus will be complete or 100 per cent. Intermediate stages in the decay of electrotonus will be represented by points that fall between the above limits. If the line through these experimental points is projected back to the half maximal constant current intercept the magnitude of the decay of the electrotonic state is expressed as the percentage of a half maximal test shock that would sum with a constant current, which was originally half maximal in strength, to produce a half maximal response. In percentage the value is the same for other constant current strengths since it has been shown that the summation curve is essentially linear.

The responses of excised nerves to subrheobasic constant currents of varied durations may now be considered. Figure 2 (excised nerve) and tables 1 and 2 give details of the responses of excised nerve as the strength of the constant current and its duration are varied. As early as 1 msec. after the constant current make there is some decay of the electrotonic state in excised nerves as is evidenced by the failure of the electrotonus to reach its theoretically maximal value. From figure 1 the time required for the electrotonus to decay to one-half its maintained level (level at 12 msec.) can be measured. These times are at the cathode for 50 per cent of rheobasic constant current 2.7 msec., for 75 per cent of rheobasic current 2.3 msec., and for 90 per cent of rheobasic current 2.2 msec. At the anode the corresponding times are 2.8, 1.8 and 2.0 msec.

Study of figures 1 and 2 and tables 1 and 2 shows that these excised nerves exhibit excitability changes in the presence of subrheobasic constant currents which are comparable to those found by Erlanger and Blair (1932). The decay of electrotonus may be considered equivalent to the accommodation of Hill (1936) and is never complete. Neither are the mirror image excitability changes at the anode and cathode, required by the theories (Rashevsky, 1933; Monnier, 1934; Hill, 1936) to be found.

The cathodal decay of electrotonus is always greater than the anodal. In as much as electrotonus has been shown to be affected by temperature (Eichler, 1933), low temperatures (about 5°C.) decreasing it, the temperature has been maintained in all experiments at about 22°C. Also the

TABLE 1

Accommodation in excised nerve in relation to the constant current strength and time of current flow

CONSTANT CURRENT STRENGTH IN RHEOBASES	TIME AFTER CONSTANT CURRENT MAKE IN MSEC.	PER CENT ACCOMMODATION	
		Cathode	Anode
0.5	1.0	18	24
0.75	1.0	26	26
0.9	1.0	12	22
1.0	1.0	6	30
0.5	12.0	70	64
0.75	12.0	66	58
0.9	12.0	72	50
1.0	12.0	74	54
0.5	1,000.0	70	64
0.75	1,000.0	86	66
0.9	1,000.0	82	50
1.0	1,000.0	86	54

TABLE 2

Excised nerve showing the course of accommodation in relation to the duration of the current

This is a case of very marked accommodation

CONSTANT CURRENT STRENGTH OF RHEOBASES	TIME AFTER CONSTANT CURRENT MAKE IN MSEC.	PER CENT ACCOMMODATION	
		Cathode	Anode
0.8	1.0	0	10
0.92	1.0	4	10
0.8	8.0	68	44
0.92	8.0	83	44
0.8	1,000.0	100	72
0.92	1,000.0	100	100

time constant of accommodation (Solandt, 1936; Katz, 1936), and the blood calcium (De Boer, 1917), are markedly influenced by temperature. The maintenance of the temperature at a nearly constant level should materially reduce variations of these factors. Since the temperature

maintained was relatively high the development of both electrotonus and accommodation should be favored (Hill, 1936; Solandt, 1936).

It should be emphasized that the data thus far presented deals with the responses of excised nerves not washed or soaked in any physiological solution. These nerves show decay of electrotonus to a marked extent although subjected to no alteration of ionic environment other than that induced by removal to the moist chamber. The extent to which pH and factors dependent on it are altered is unknown and not subject to control. The differences in the responses of these nerves and the circulated ones

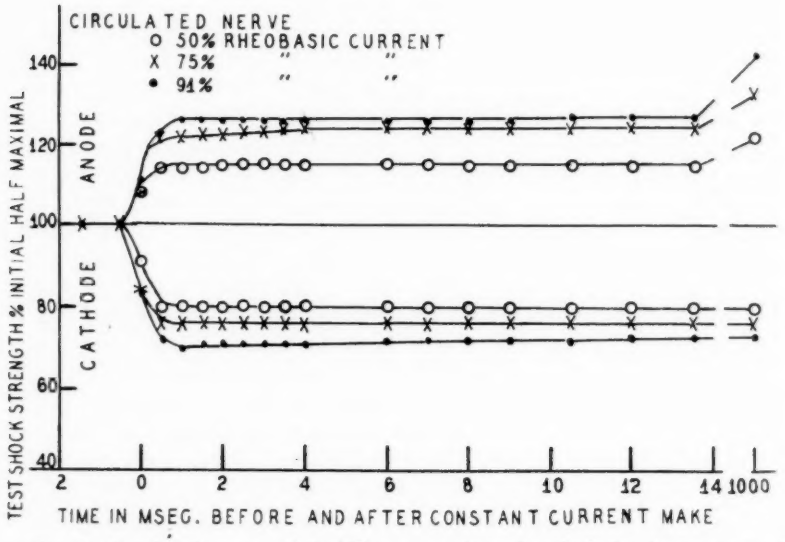

Fig. 3. Circulated nerve. Irritability at anode and cathode in the presence of 50 per cent, 75 per cent and 91 per cent of rheobasic constant currents. Compare with figure 1.

to be discussed next indicate that no small amount of change takes place in the nerve upon removal from the normal ionic medium maintained in the living animal.

Nerves provided with circulation. Studies were made on nerves in situ with the circulation maintained as nearly normal as was possible under experimental conditions. Although preparation for stimulating and recording led to some hemorrhage it was possible to maintain a reasonably good circulation when the operational procedures were kept at a minimum. The responses of circulated nerves to constant currents are shown in figures 2, 3 and 4. The details of the time course and magnitude of the

electrotonic state are shown in figure 3 which should be compared with figure 1 where similar data are presented for excised nerves. Figures 2, 3 and 4 demonstrate forcibly that there is essentially no decay of the electrotonic state, that is, no accommodation, in circulated nerves subjected to subrheobasic constant currents. Figure 3 shows clearly the marked depression of excitability developed at the anode when currents

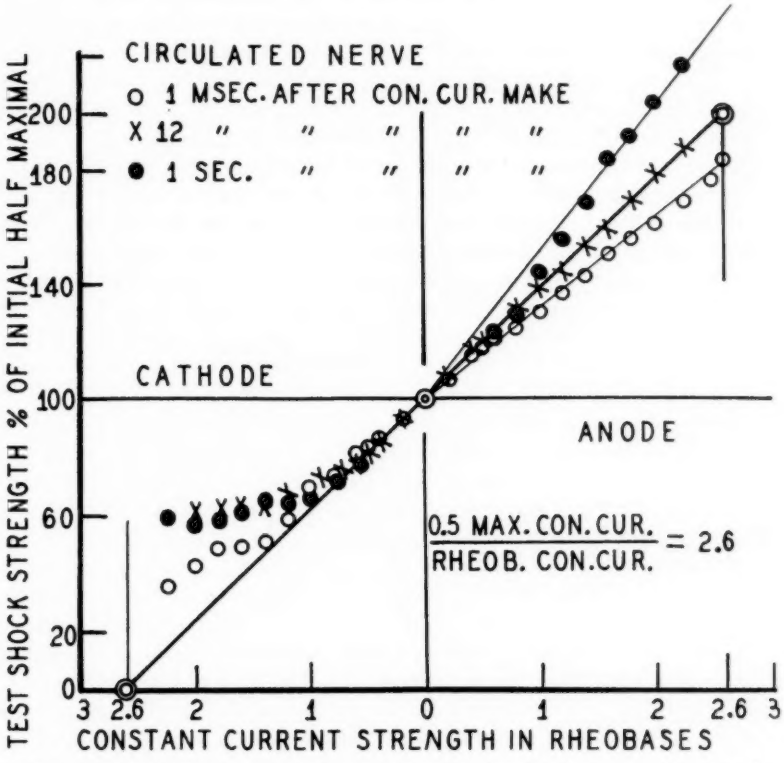

Fig. 4. Circulated nerve. Accommodation in circulated nerve. See text for detailed discussion.

of long duration are applied. Study of the figures will reveal other difference in the magnitude and time relations of the electrotonic states of the anode and cathode. An increase of the excitability at the cathode with currents of long duration sometimes occurs but this is never equal in magnitude to the anodal depression developed in the same nerve under the same conditions. Figure 4 gives data for currents increasing from subrheobasic to half maximal in strength. Compare the linear effects

produced at the anode by currents over this range with the effects of the same currents at the cathode. The deviation from the linear relation at the cathode is to a large extent owing to the fact that the test stimulus falls in the refractory period of the nerve which follows its response to the constant current. Such interference by the refractory period begins as soon as the constant current is of threshold strength for any fiber in the nerve trunk. Since the response of these circulated nerves to the constant current is repetitive the interference persists as long as the constant current is applied. Therefore, the test shock is increased in strength and the extent of the increase is largely determined by the point at which the shock falls in the refractory period following a response to the constant current. No responses have been obtained from circulated nerves on the break of constant currents allowed to flow for one second or longer. Figure 5 shows the excitability changes following the interruption of constant currents of 1 sec. duration and supplies a reason for the absence of the break response. The anodal depression is seen to disappear rapidly at first and then much more slowly without, however, passing through a stage of increased excitability which could give rise to a break response. This is in marked contrast to the readily obtained break response of excised nerves and is in itself evidence for a negligible accommodation in circulated nerves. Note the marked differences in the time courses of decay of the electrotonic state at the anode and cathode (fig. 5).

All the data obtained from circulated nerves indicated that when the circulation to the nerve was adequate no decay of the electrotonic state would occur. The experiments of Liesse (1938a, b) point in the same direction. In a few circulated preparations where no accommodation occurred (curves identical with fig. 3) ligation of the blood vessels to the nerve brought about a response identical with that of excised nerves (fig. 1) in 10 to 30 minutes.

Excised nerves soaked in various media. The effects of soaking excised nerves (see figs. 1 and 2 and tables 1 and 2) in Ca^{++} and K^{+} before applying the constant current are summarized in table 3. Soaking the nerve in Ca^{++} rich Ringer brings about a response very like that of the circulated nerve except that the anodal depression is frequently much more pronounced. The decay of electrotonus, accommodation, is much less than in unsoaked excised nerves. Compare these effects of added Ca^{++} with the results of P. H. and M. Benoit (1937) on the removal of Ca^{++} by citrate injections in pithed frogs. An opposite effect is obtained when the nerves are soaked in K^{+} rich Ringer. The magnitude of the decay of electrotonus, accommodation, is increased and the time to half accommodation is shortened. In a freshly excised nerve the time to half accommodation was 3.8 msec. at the cathode and 3.7 msec. at the anode. Soaking this nerve in excess K^{+} reduced these times to 2.6 msec.

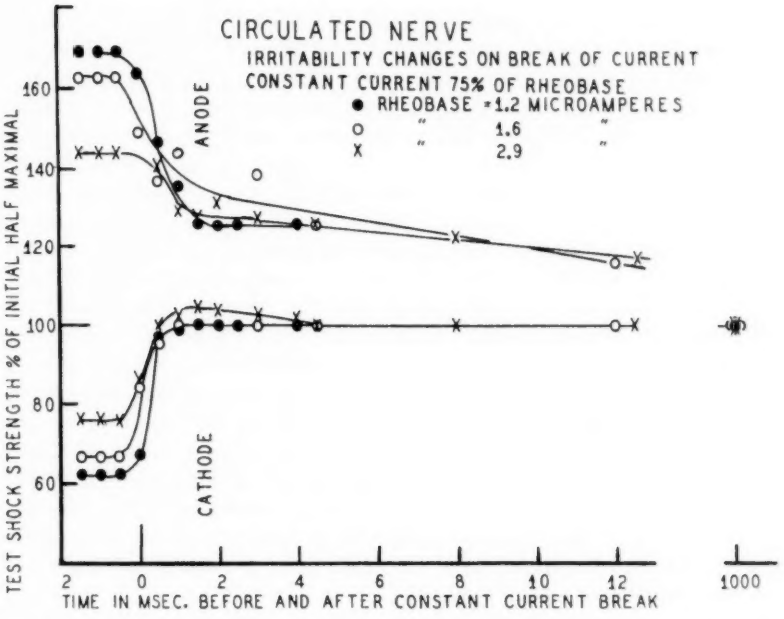

Fig. 5. Circulated nerve. Irritability changes on the break of a constant current of one second duration in circulated nerve.

TABLE 3

Comparison of the per cent accommodation in unsoaked excised nerve and in the same nerve after soaking in Ringer containing excess Ca^{++} or excess K^{+}

TIME AFTER CONSTANT CURRENT MAKE IN MSEC.	PER CENT ACCOMMODATION		PREVIOUS TREATMENT OF THE NERVE
	Cathode	Anode	
1.0	40	44	Excised nerve unsoaked
12.0	60	60	
1,000.0	76	60	
1.0	12	12	Same nerve after soaking in excess Ca^{++}
12.0	0	0	
1,000.0	−16	−120	
1.0	20	58	Nerve from opposite side after soaking in excess K^{+}
4.0	92	76	
12.0	92	82	
1,000.0	100	100	

at the cathode and 2.7 msec. at the anode. The exact rôle of potassium is difficult to determine since Chweitzer (1935) and Bouman (1937) have found that it decreases catelectrotonus while Hill and Solandt have found that it decreased the time constant of accommodation. It is not possible to assign values to these two effects from the data of the present experiments.

It is probable that the Ca^{++}/K^{+} ratio is more important in the normal functioning of the nerve than the absolute amounts of either. Lehmann (1937a and 1937b) has found a close correlation between the pH and the Ca^{++}/K^{+} ratio. The pH was not controlled in these experiments and the extent to which alterations of pH may have altered the Ca^{++}/K^{+} ratio is unknown. The data obtained on the effects of Ca^{++} on the threshold for electrical stimulation are in agreement with the findings of Lehmann. Neither Hill's normal Ringer, used by Solandt (1935), nor that of Kato (1934) maintained the nerve so that the electrotonic phenomena were the same as in the circulated nerve. It is probable, therefore, that the circulation maintains the pH and the Ca^{++}/K^{+} ratio about the nerve so that decay of electrotonus, accommodation, does not occur. Similarly the removal of the nerve from the body to a moist chamber alters the pH and the Ca^{++}/K^{+} ratio in such a way that accommodation is the usual response.

Conclusions. In experiments performed on the frog's sciatic nerve the excitability of the alpha fibers, during the passage of subrheobasic constant currents, has been investigated by the test shock method (Erlanger and Blair, 1932a, b), using the half maximal alpha action potential as the index of the nerve's activity. It has been shown that with a particular duration of the constant current there is a linear or nearly linear relation between the strength of the required test shock and the strength of the constant current (figs. 2 and 4). In view of this relationship the variations of excitability are adequately measured by variations of the required test shock. The excitability of the nerve at any moment as measured by the test shock strength may conveniently be expressed as percentage of the test shock strength which, when applied alone, gives a half maximal response. The excitability so measured is proportional to $(j\text{-}\epsilon)$ of Rashevsky (1933, 1938) or to (U-V) of Hill (1936).

The alterations of excitability that follow the application of subrheobasic constant currents have been measured in excised and in circulated nerve. The excitability change attains a maximal level shortly after making the constant current and, in the excised nerve, returns toward the resting level as the duration of the current is prolonged (figs. 1 and 2 and tables 1 and 2). This return toward the resting condition has been called the "decay of electrotonus" but it may equally well be considered as inhibition, adaptation or "accommodation" in the sense of Hill (1936). The

magnitude of the decline of excitability is a measure of the amplitude of accommodation and the magnitude of the final level of the altered excitability is a measure of the maintained electrotonus. Since, in freshly excised nerves, there was always some maintained electrotonus and since there were striking differences in its magnitude at the anode and cathode the complete accommodation and the mirror image relations of the anode and cathode, demanded by the two factor theories (Rashevsky, Monnier, Hill), were not found.

In circulated nerves the electrotonic state was maintained throughout the time of constant current passage. Usually it increased in magnitude with long durations particularly at the anode. This indicated that in circulated nerve accommodation is zero. The repetitive response of circulated nerves to suprarheobasic constant currents and the absence of a break response support this concept. Since it can be concluded from these observations that accommodation is essentially a property of excised, uncirculated nerves the two factor theories of excitation, which can all be shown to be identical and to require 100 per cent accommodation, must be altered to include an added term. This term in I (equation 1) has always been made zero theoretically and occasionally experimentally (Hill, Solandt, Lucas), and has been justified by the liminal gradient experiments of von Kries (1884a, b) and Lucas (1907). Since the present experiments and those of Liesse (1938a, b) suggest that the liminal current gradient and accommodation are properties of excised nerve the two factor theories must now include the term in I in order to explain the responses of both excised and circulated nerve. Under these conditions Hill's methods for measuring the time constant of accommodation (λ) do not apply. These experiments, therefore, strongly suggest the need for the investigation of other aspects of nervous activity in the presence of an adequate circulation before the data obtained from excised nerve is used too freely in interpreting normal nervous function.

SUMMARY

The excitability of the alpha fibers in the frog's sciatic nerve during the passage of a subrheobasic constant current has been measured by determining the strength of a short test shock necessary to evoke half maximal alpha spike potentials. As found by other investigators, the alteration of excitability in excised nerves is maximum at about one millisecond after the application of a constant current and usually falls to a steady value other than zero. The decrease and the final value of excitability are measures of the amplitude of accommodation and electrotonus respectively.

Excised nerves not exposed to Ringer's solution show both accommodation and electrotonus. Ringer's solution containing excess Ca^{++}

reduces the amplitude of accommodation and may make it zero or negative, but excess K^+ increases the amplitude of accommodation and shortens the time to half accommodation.

Nerves with intact circulation in spinal animals show a negligible accommodation up to one second after application for currents 50 to 90 per cent of rheobasic. After the circulation is blocked or during anesthesia, the amplitude of accommodation becomes comparable to that of excised preparations.

In all preparations the effects at anode and cathode are not mirror images of each other. When present, accommodation is always less at the anode than at the cathode, and negative accommodation in circulated preparations and excised preparations subjected to excess Ca++ is more marked at the anode.

If other experiments also demonstrate that accommodation is negligible under nearly normal physiological conditions, it should be considered as an abnormal alteration of electrotonus, excitability or threshold which is characterized by an amplitude as well as a time constant. The two factor theories of excitation allow this viewpoint if the minimal gradient and allied phenomena may be classified as abnormal.

The author wishes to express to Dr. K. S. Cole appreciation of his pertinent criticism of this paper and to extend to the Department of Physiology thanks for the facilities provided during the course of the work.

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THE REDUCTION OF THE BLOOD PRESSURE OF HYPERTENSIVE DOGS BY THE ADMINISTRATION OF RENAL EXTRACT[1, 2]

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The pressor effects of renin can be diminished by the previous administration of an extract of the kidneys and such extracts also reduce the blood pressure of rats with hypertension caused by the removal of renal tissue (1). The purpose of the present study was to determine whether a similar effect could be induced in other types of renal hypertension. For this purpose dogs with hypertension due to renal ischemia (2) were employed. Such animals differ from the hypertensive rats previously studied in several respects: the amount of functioning renal tissue is greater, and the duration of the hypertension is longer in such dogs than in the rats; renal ischemia is present in the dogs but absent, according to the best available evidence (3), in the rats.

METHODS. Hypertension was induced in a series of dogs[3] by the application of clamps to the renal arteries according to the procedure described by Goldblatt, Lynch, Hanzal and Summerville (2). The mean blood pressure was measured directly on the unanesthetized animals by puncture of the femoral artery with a needle attached to a mercury manometer. In all instances numerous daily measurements were made before, during and after treatment.

Renal extract was purified and concentrated according to the procedures described elsewhere (4). A total of eight dogs were treated by intraperitoneal or intravenous injection of the concentrated renal extract, each animal receiving several injections during the period of one to four days and treatment then being withheld for one or more weeks. Most of these animals received several such courses of treatment. Ten animals

[1] This work was aided by grants from the Josiah Macy Foundation and from Mr. Joe Werthan.

[2] Reported before the Cleveland Academy of Medicine, September 15, 1939.

[3] We are indebted to Dr. Alfred Blalock for his kindness in allowing us to use several of his animals and for supplying us with the facilities of his laboratory for the operations on the remaining dogs.

were also given renal extract either with the food or in most instances by stomach tube three to five times daily for a period of one day to two weeks, the blood pressure being measured one or more times per day during and after the course of therapy.

All the extracts used in the present work were first assayed on rats. As a preliminary unit we have utilized that amount of extract which will cause a reduction of 50, 35 and 25 mm. in rats having initial blood pressures of 200, 175 and 150 mm. of mercury, respectively. The amount of extract administered to each dog varied from 5 to 200 times this unit. In general, it was found that the dog requires about thirty to fifty times as much extract as does the rat in order to produce a comparable reduction in blood pressure. This is in accord with what one would anticipate from the relative surface areas of the two species.

Results. *Parenteral therapy.* Each of the eight dogs which received renal extract by the intravenous or intraperitoneal route exhibited a decline in blood pressure. This appeared within 6 to 24 hours after instituting therapy and persisted for several days after treatment had been discontinued. The degree of decline in blood pressure varied markedly in different animals, the greatest diminution being approximately 60 mm. and the smallest 20 mm. In most instances the pressure began to increase within two to three days after the cessation of therapy, increased rapidly to a level somewhat less than that of the control period and then gradually rose during one or more weeks, the control level being finally reached in some animals but not in others.

In most instances the treated animals exhibited untoward symptoms. On several occasions severe collapse occurred but more frequently the animals displayed weakness, apathy and vomiting. Two of the animals treated parenterally displayed only the mildest toxic symptoms, but the declines in blood pressure were just as great in these animals (fig. 1A) as in those showing more striking toxic effects.

It is well known that all tissue extracts may contain toxic and depressor substances. The immediate decline in blood pressure occurring within a few hours after the administration of renal extract might justifiably be ascribed to such nonspecific substances and until the preparations can be further purified there can be no certainty as to whether the observed diminution in blood pressure was dependent on the administration of toxic agents or of some specific renal substance which is lacking in hypertensive animals. For these reasons the parenteral route of administration was abandoned when it was found that oral administration was efficacious in reducing the blood pressure, for it would seem that the disadvantage of having to use larger amounts of extract was more than offset by the reduced likelihood of obtaining non-specific depressor effects.

Oral therapy. Fourteen courses of treatment were given to ten dogs.

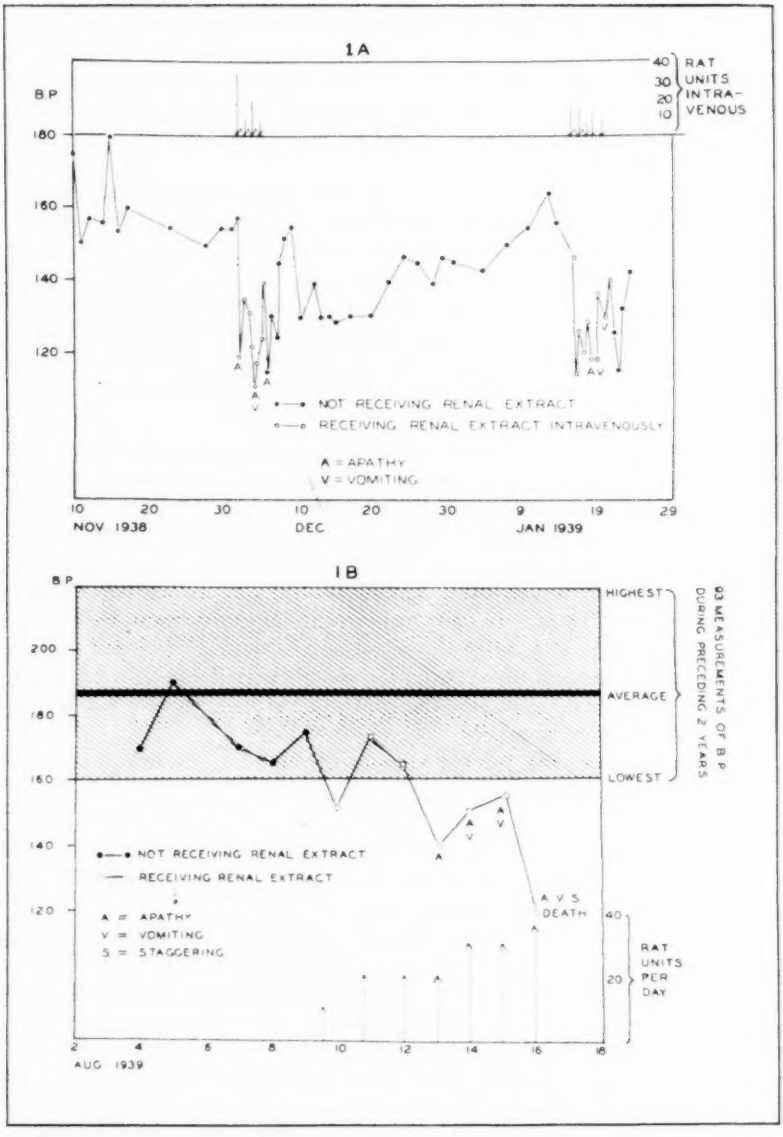

Fig. 1A. Intravenous injection of renal extract was followed by a decline in mean blood pressure of approximately 40 mm. After treatment ceased the blood pressure rose slowly and declined again when therapy was resumed. During treatment the animal vomited occasionally and was slightly apathetic.

Fig. 1B. Slight reduction of blood pressure following the oral administration of renal extract was accompanied by no symptoms. As the dose was increased and the pressure declined further uremia and death occurred.

In ten instances a decline of blood pressure of 20 mm. or more below the average pre-treatment level was observed. In one experiment the blood pressure declined but so slightly (15 mm. of mercury) as to be of doubtful significance. In the remaining three instances no appreciable change in pressure occurred. These three animals received each a total dose of less than twenty units. The total dose in all of the other animals was fifty units or more.

We had hoped that oral therapy, if successful in reducing the blood pressure, would not be attended by toxic effects. However, such was not the case, administration of large doses of extract often inducing undesirable symptoms. The usual phenomena displayed were apathy and weakness. One of the ten dogs vomited frequently, five others vomited occasionally during treatment, and the remainder did not vomit. One of the dogs which displayed marked decline in blood pressure developed uremia and died. In two other animals which displayed a decline in blood pressure under treatment and well marked weakness and apathy, measurements of the non-protein nitrogen of the blood revealed minimal evidence of renal insufficiency. It was observed that those animals which had the highest initial blood pressures and hypertension of the longest duration, usually displayed the most marked symptoms of intoxication during treatment. Three dogs with mild hypertension exhibited no toxic symptoms at all either during or after treatment, in spite of a well marked diminution in blood pressure. However, the toxic symptoms were not entirely due to decline in blood pressure for two of the three animals which received minimal doses and had no decline in pressure also displayed mild untoward symptoms.

In figures 1B, 2A and 2B are reproduced typical experiments showing the changes in blood pressure following oral therapy with renal extracts. The control values cited on the figures before therapy were in the range of those observed for many weeks previous to the therapy and show the general range of unavoidable variation in the daily readings.

The dog of figure 1B promptly responded to the administration of extract with a reduction from an average of about 175 mm. to 150 mm. without any obvious detrimental effect on the animal. When the administration of the extract was continued and larger doses were used a further fall in blood pressure to the normal value of 120 mm. occurred on the seventh day of treatment. At this time manifestations of uremia were observed and the animal died 24 hours later.

In figure 2A is shown the result of an experiment in which the animal showed symptoms of apathy and vomiting when the blood pressure had declined from its original value of 190 to 163 mm. When therapy was discontinued and the pressure rose to 175 the symptoms disappeared

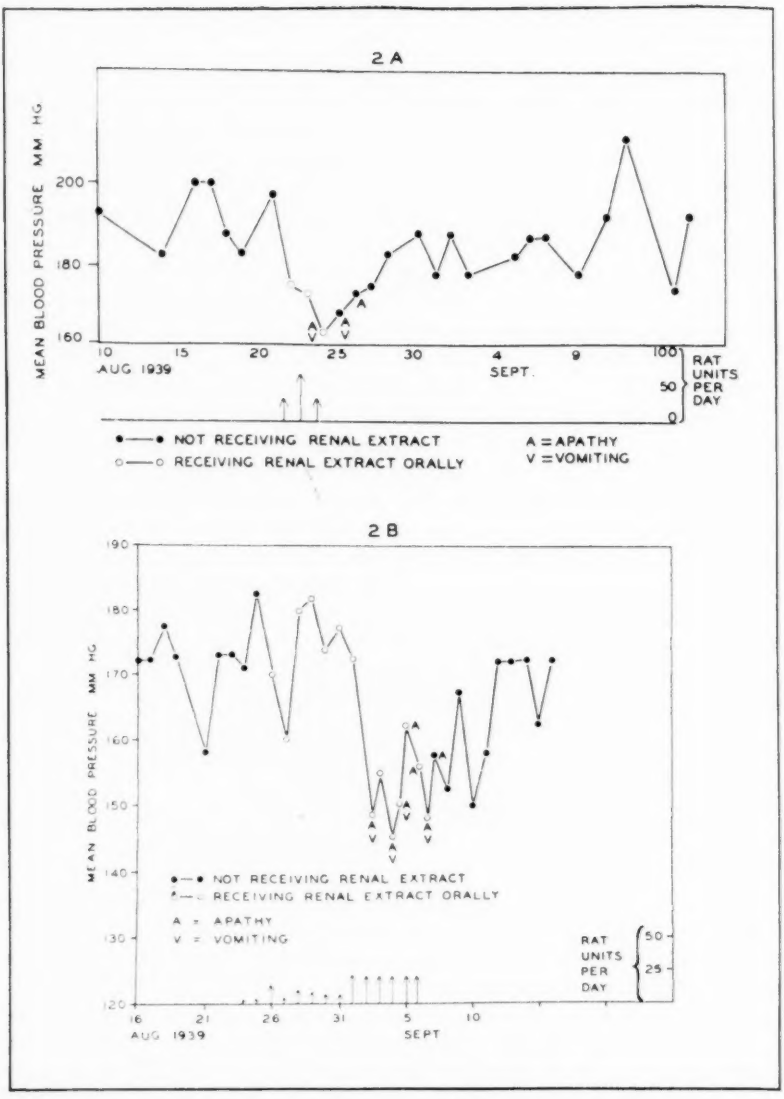

Fig. 2A. The administration of renal extract by mouth was followed by a prompt although slight decline in blood pressure and by weakness and vomiting.

Fig. 2B. Large doses of a relatively inactive extract produced no symptoms and no decline in blood pressure. When a more active extract was administered the blood pressure declined about thirty millimeters and mild untoward symptoms occurred. After treatment was discontinued the blood pressure gradually rose to the original level.

and the animal remained symptom-free on the following days, despite the reduction in blood pressure below the pre-treatment values.

Figure 2B reproduces the results obtained on an animal to which large doses of relatively inactive extracts (as assayed on rats) were first administered without any appreciable change in the blood pressure. The volume of extract administered and its content of contaminating substances present were essentially those present in the potent extracts. The fact that no symptoms were induced by this procedure would indicate that these are—in part—results of the decline in blood pressure rather than entirely due to any impurity present in the extract.

Discussion. The observations which have been cited indicate clearly that the administration of properly prepared and concentrated extracts of normal renal tissue either parenterally or orally may cause a definite decline in blood pressure of dogs with hypertension due to renal ischemia. However, since the diminution obtained by the parenteral route of administration was accompanied by some abnormal symptoms in all of the animals and by severe illness in some of them, there is a definite possibility that the effects observed in this group of experiments may have been due to toxic and non-specific depressor substances. Such an objection is, however, less applicable to those experiments in which the renal extract was administered orally. Here again certain untoward manifestations sometimes occurred. There is, however, reason to believe that these undesirable symptoms were partly the result of the decline in blood pressure rather than entirely due to any toxic effect of the extracts.

In animals with highly elevated blood pressure any appreciable drop in this pressure must result in a deficient blood supply to the kidney, due to the presence of the clamp. A further factor to be considered is the generalized arteriolosclerosis which is known to follow long-standing experimental arterial hypertension. A subsequent reduction in blood pressure in such an animal would necessarily result in ischemia of the tissues. The effects of this ischemia in the nervous system and other vital organs would account for the untoward symptoms observed when the pressure was appreciably lowered in animals with long-standing hypertension of high degree.

One point merits especial emphasis. The phenomena described in the dog—namely, the decline in blood pressure to a normal level without untoward symptoms when the hypertension was mild, but severe symptoms and even death associated with the decline in pressure when the hypertension was more severe—are quite like those which we have already described in the rat (1). This similarity in response constitutes further evidence that the hypertension due to renal ischemia and that due to a deficiency of renal tissue are results of some common underlying renal disorder.

SUMMARY

A series of 18 hypertensive dogs were treated with renal extracts. The blood pressure of these animals was appreciably reduced, normal values being attained in some cases. Severe untoward symptoms accompanied this reduction in many cases. The bearing of these findings on certain aspects of the problem of renal hypertension has been discussed.

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THE EFFECT OF CASTRATION AND ANDROGEN THERAPY ON CREATINE AND CREATININE EXCRETION IN MONKEYS[1]

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The relation of creatine excretion to age and sex differences in man, and to species and sex differences in animals, is generally known. Children of both sexes and adult women excrete creatine, even while on a creatine-free diet, while adult men do not. The capacity to retain exogenous creatine is much greater in men than in women or children (Hunter, 1928).

In view of the above findings and those of Read (1921) and McNeal (1922), who have shown that eunuchs excrete creatine, interest has been focussed on the problem of whether a hormone or a lack of a hormone is responsible for the creatinuria. However, Tsun-Chee-Shen and Lin (1927) observed a creatinuria in but 1 of 9 eunuchs.

Kun and Peczenik (1932) have induced a creatinuria in rats when castrated post-puberally and which was subsequently suppressed by a urinary androgen (Proviron). Buhler (1933) has been successful in reducing the creatinuria occurring in alleged sexual hypofunction by the administration of androgen. Remen (1932) has shown that creatine retention is impaired in old age and castration. Retention can be improved by androgen or gonadotropic factor (Nitzescu and Gontzea, 1937).

Recently, Kenyon et al. (1938, 1940) have markedly reduced the creatinuria occurring in a eunuchoid, as a result of daily ingestion of creatine, by giving 25 mgm. testosterone propionate per day. In rats (Coffman and Koch, 1940) no creatinuria occurs after castration, nor is the capacity to retain exogenous creatine reduced below that of normal rats. The creatinuria, however, which is induced by feeding creatine, can be greatly reduced by the administration of testosterone propionate.

Koven, Beard (1939, 1940), Pizzolato and Beard (1939) have performed a long series of experiments on rats. They have observed a creatinuria

[1] Aided in part by a grant from the Rockefeller Foundation, administered by Dr. Philip E. Smith.

[2] University Research Fellow, College of Physicians and Surgeons, Columbia University, 1938–1939.

after castration in both males and females. Curiously enough they report that a creatinuria was also produced by injecting testosterone, estrogen, anterior pituitary extracts, cortin, saline and water.

Tsun-Chee-Shen (1927), Sandberg, Perla and Holly (1938) have failed to observe any change (in dogs and rats) in creatine excretion as a result of castration. Kochackian and Murlin (1935) did not observe a creatinuria in castrated dogs on a creatine-free diet, but did when the dogs were on a diet containing creatine.

EXPERIMENTAL. The experiments reported here are concerned with the relation of androgen and castration to creatine metabolism in the monkey. Nine male rhesus monkeys (Macaca mulatta) were used in this study, with each animal serving as its own control. They were kept in individual metabolism cages and on a diet practically free of creatine (consisting of fresh fruits and vegetables and a boiled egg thrice weekly). Twenty-four hour samples of urine were analyzed for creatinine and creatine according to the methods of Folin (1914) and modification of Benedict and Myers (1907) respectively. Each determination was done in duplicate; the creatinine values are expressed in milligrams and the creatine in milligrams creatinine.

The animals were castrated under ether anesthesia with sterile technique.

Creatine retention tests were performed in the following manner: 227 mgm. creatine hydrate (200 mgm. creatine), dissolved in sterile saline, were injected intraperitoneally. Two specimens of urine were collected, one after 24 hours and the other after 48 hours, and the total amount of creatine excreted was expressed in percentage excretion. On the average, two creatine retention tests were done for each procedure.

Testosterone propionate and estradiol benzoate were injected intramuscularly in sesame oil, and the human chorionic gonadotropin (PU.),[3] in sterile saline, was given subcutaneously.

Since glucose and acetone bodies interfere with the Jaffé reaction, the urine was tested qualitatively for these substances at various intervals, but in no case were these substances found.

Very careful records of the food intake were made during a considerable part of this study, but no correlation could be made between the creatine and creatinine values and the type and quantity of food, and no effect was observed after egg feeding.

Creatine excretion in immature monkeys. Immature monkeys normally excrete creatine as well as creatinine. Three of the four monkeys showed a creatinuria while the other excreted creatine for only two days during

[3] Perandren was supplied by Ciba Pharmaceutical Products, Inc., through the courtesy of Mr. R. C. Mautner; Progynon B by Schering Corporation, through the courtesy of Dr. Erwin Schwenk; and Follutein by E. R. Squibb & Sons, through the courtesy of Dr. J. A. Morrell.

the control period. When given creatine retention tests, these animals did not retain more than 30 per cent and excreted the remainder.

The injection of testosterone propionate or human chorionic gonadotropin results in the abolition of the creatinuria, and increases the capacity to retain exogenous creatine (table 1).

Monkeys 1 and 2 (3.8 and 3.3 kilo) were both immature monkeys with inguinal testes. Monkey 1 excreted during a control period of 13 days a daily average of 115 mgm. creatinine and 17 mgm. creatine; monkey 2 was creatine-free except for 2 days, and excreted 110 mgm. creatinine. However, the average for two retention tests on each animal showed that neither retained more than 31 per cent and excreted the remainder. Five milligrams of testosterone propionate were then administered to

TABLE 1

The effect of androgen treatment on immature monkeys

MONKEY	CONTROL PERIOD				INJECTION PERIOD			
	Days	Creatinine	Creatine	C.R.T. (excretion)	Days	Creatinine	Creatine	C.R.T. (excretion)
		mgm.	*mgm.*	*per cent*		*mgm.*	*mgm.*	*per cent*
1	13	115	17	70	15	122	3	33
	6	142	30					
2	13	110	0	71				
3	13	147	25		10	153	0	
	5	162	35					
4	8	236	38		7	216	0*	

* 400 r.u. Follutein on alternate days.

no. 1 for 15 days, resulting in a gradual abolition of the creatinuria. The average for two retention tests resulted in 33 per cent excretion.

After cessation of androgen therapy, the creatinuria reappeared (33 mgm. per day) within 96 hours. (Fig. 1 shows the daily excretion values for monkey 1.)

Monkey 3 (4.8 kilo) exhibited a creatinuria for 11 days during a 13 day control period; 147 mgm. per day of creatinine and 25 mgm. per day of creatine were excreted. Two milligrams of testosterone propionate per day for 10 days abolished the creatinuria (except for 1 day) which subsequently returned within 48 hours after cessation of treatment (fig. 2).

Monkey 4 (6.5 kilo) excreted a daily average of 236 mgm. of creatinine and 38 mgm. of creatine during a control period of 8 days. This animal was of adult size and had scrotal testes. Since it showed a creatinuria,

one testis was removed for histological study. It resembled that of an immature monkey, having no sperm, few spermatocytes, and as far as could be determined, very few interstitial cells of Leydig. Four hundred rat units of human chorionic gonadotropin (Follutein) givin on alternate days caused a complete disappearance of the creatinuria within 72 hours. The remaining testis was then removed and the creatinuria set in again.

It was then attempted to see whether estrogen therapy had any effect in reducing the creatinuria which occurs in immature monkeys. Monkey 1 was injected with 100 r.u. per day of estradiol benzoate but there was no significant reduction in the creatine output. Creatine retention tests showed an average of 72 per cent excretion.

Creatine excretion in mature monkeys. Of 5 mature monkeys studied, 2 were entirely creatine-free during the control period; 1 had a creatinuria

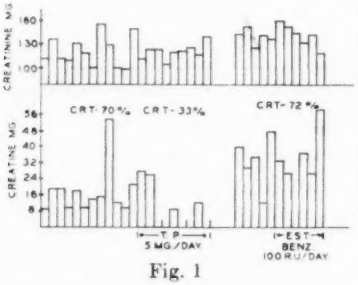

Fig. 1

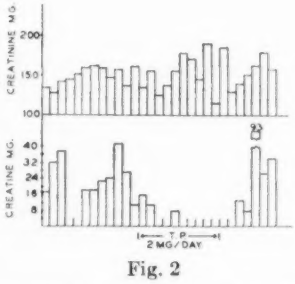

Fig. 2

Fig. 1. Monkey 1. Each vertical bar represents a 24 hour sample. C.R.T., creatine retention test expressed as per cent excretion. *T.P.*, testosterone propionate. *Est. benz.*, estradiol benzoate.

Fig. 2. Monkey 3. Same symbols as in figure 1.

one-third of the time, while the others excreted creatine about one-half the time. All of the animals, however, reacted similarly to exogenous creatine by retaining most of it. After castration the creatinuria became more intense in all the animals except in those which excreted the most creatine during the control period.[4] The ability to retain exogenous creatine was now impaired.

The injection of 5 mgm. per day of testosterone propionate within 2 to 5 days completely abolished the creatinuria which ensued as a result of castration. The animals were now tested for their creatine retention

[4] It is interesting to note that monkey 7 and monkey 9 both had inguinal testes, which were small, aspermic and showed few interstitial cells of Leydig. These animals were castrated because of the fact that they showed excellent retention of exogenous creatine. Although no. 9 was not affected by castration, testosterone propionate abolished the creatinuria in both.

capacity and retained even more than during the control period (table 2). Although two of the animals were not affected by castration, the androgen completely abolished the creatinuria in all the monkeys.

TABLE 2

The effect of castration and androgen treatment on creatine and creatinine excretion in mature monkeys

MONKEY	CONTROL PERIOD				CASTRATE PERIOD				INJECTION PERIOD			
	Days	Creatinine	Creatine	C.R.T. (excretion)	Days	Creatinine	Creatine	C.R.T. (excretion)	Days	Creatinine	Creatine	C.R.T. (excretion)
		mgm.	*mgm.*	*per cent*		*mgm.*	*mgm.*	*per cent*		*mgm.*	*mgm.*	*per cent*
5	18	204	*	32	14	185	25	100	7	189	0†	15
					5	199	34		15	205	0	
					16	198	23	78	9	199	0	22
					8	219	72	100				
6	12	146	0	24	17	149	15	44	13	148	0	13
					10	160	19	52				
7	14	88	14	11	19	132	14	20	12	138	0	0
					9	146	42	77				
8	14	175	0	25	15	170	24	60	8	161	0	15
					8	177	27	44				
9	14	165	19	16	17	172	17	24	9	159	0	20
					6	199	18	13				

* Intermittent creatinuria.

† Does not include days when creatine values were declining.

C.R.T. = creatine retention test.

TABLE 3

The effect of estrogen treatment on creatine and creatinine excretion

MONKEY	DAYS	CREATININE	CREATINE	C.R.T. (EXCRETION)	DOSE
		mgm.	*mgm.*	*per cent*	*r.u. per day*
1	6	142	30	72	100
5	8	176	45		100
5	6	231	38	79	200

After cessation of androgen therapy the creatinuria reappeared. Of the two monkeys in which there was little change in creatine excretion immediately after castration, one (no. 7) later developed an intensified creatinuria. During this second castrate period the animals (except no. 9) had an impaired capacity to retain exogenous creatine.

In figures 3 and 4 is the record of the daily output of creatinine and creatine for monkeys 5 and 6.

The above procedure of alternating injection periods with castrate periods was repeated three times on monkey 5, during a period extending over 9 months, with similar results each time. The administration of 100 and 200 r.u. of estradiol benzoate had no effect on the creatine excretion or creatine retention tests.

None of the procedures performed—castration, androgen, gonadotropic or estrogen therapy—had any significant effect upon the excretion of creatinine.

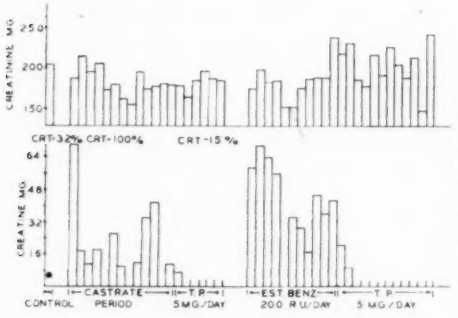

Fig. 3

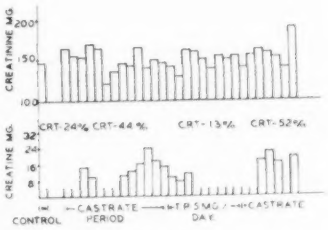

Fig. 4

Fig. 3. Monkey 5. *Intermittent creatinuria, control period is an average of 18 days. Same symbols as figure 1.

Fig. 4. Monkey 6. Control period is an average of 12 days. Same symbols as in figure 1.

Discussion. It appears clear from the results presented above that the creatinuria which usually occurs in young monkeys can be abolished by the administration of androgen. These monkeys do not tolerate exogenous creatine to any great extent and excrete most of the injected creatine. However, after androgen therapy the capacity to retain creatine is enhanced. Older monkeys may or may not exhibit a creatinuria, but when exogenous creatine is administered intraperitoneally most of it is retained. After castration a creatinuria appears, or is usually intensified if present during the control period. During this castrate period the animals usually do not retain creatine to as great an extent as previous to castration. Testosterone propionate injections result in the disappearance of the creatinuria in all cases, whereas estrogenic therapy, in the doses used, is ineffectual.

Immature or castrated monkeys do not store or metabolize creatine as well as do mature or androgen-treated animals. The reason that

children, women and eunuchs (even on a creatine-free diet) excrete creatine may be due to the fact that creatine formed from endogenous sources cannot be stored or metabolized as well as in the adult normal man.

In hyperthyroidism, muscular dystrophies, infectious diseases and many other conditions, an intense creatinuria occurs (Hunter, 1928). This may vary from 50 to 100 per cent of the creatinine values. However, the creatinuria of childhood or castration is of a much smaller magnitude, varying from 10 to 30 per cent of the creatinine values. About a month after the studies on one of the above monkeys were concluded, symptoms of tuberculosis were noted. The urinary creatinine averaged 155 mgm. per day while the creatine varied from 40 to 120 mgm. The injection of 5 mgm. of testosterone propionate daily had no effect upon the creatine excretion which continued to mount. The animal was killed and the clinical diagnosis of advanced pulmonary and generalized miliary tuberculosis was confirmed at autopsy. Thus, it would appear that the causative factors in these cases are different and the therapeutic agents effective in one may be ineffectual in the other.

It should be pointed out that impaired creatine retention occurs during hyperthyroidism, advanced muscular dystrophies and other conditions (Hunter, 1928; Milhorat and Wolff, 1938; Richardson and Shorr, 1936, and others).

Schrire and Zwarenstein (1934) have shown that in the rabbit, castration or ovariectomy results in an increased creatinine excretion which occurs after a latent period of months. However, some of the monkeys studied have been under observation for as long as 8 months after castration and yet no significant increase in creatinine excretion was noted. A human castrate has also been observed in the Squier Urological Clinic,[5] nine months after castration without treatment, and yet no significant increase in creatinine excretion occurred.

Recent work has indicated that the androgen may have an effect on general body economy as well as on the accessories. Papanicolaou and Falk (1938) have shown that injection of testosterone propionate will cause an hypertrophy of the temporal muscles of castrated male or female guinea pigs. Kenyon et al. (1939) found that androgen therapy would produce retention of Na, N, P, K, Cl, together with an increase in weight due to water held in association with salts and proteins retained. Hesser, Langworthy and Vest (1940) have reported increase in muscle work in myotonia atrophica cases with the administration of testosterone propionate.

Kenyon and his associates have discussed the general somatotropic influence of the androgen in man. Heretofore studies of the effects of androgen and estrogen have been concentrated on the sexual accessory

[5] This patient was placed at my disposal through the courtesy of Dr. G. W. Fish.

organs, but in view of the fact that testosterone has an effect on Na, N, P, Cl, K, as well as water retention, creatine excretion, muscle mass and work, blood vascular system, as well as many subjective symptoms (Steinach, Kun and Peczenik, 1937), it seems possible that the androgen has an effect which is more general than considered previously. What the specific effects on body economy are, remain unknown at this time. However, in studying the metabolic effects, the differences between the physiological and toxic effects must be distinguished, since it is known that excessive dosages of androgen and estrogen may be toxic.

I should like to express my appreciation to Dr. E. T. Engle for his invaluable advice and encouragement during this study.

SUMMARY AND CONCLUSIONS

Young male monkeys usually excrete creatine, and show impaired retention of exogenous creatine.

Injection of testosterone propionate causes an abolition of creatinuria and increases the capacity to retain creatine.

Mature monkeys may or may not exhibit a creatinuria, but retain exogenous creatine.

Castration in mature monkeys results in an increased creatinuria and impaired creatine retention.

The injection of testosterone propionate abolishes the creatinuria of castration and restores the capacity to retain creatine.

Estradiol benzoate therapy is without effect on creatine excretion.

None of the procedures reported produced any effect on the level of creatinine excretion.

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THE DISTRIBUTION OF BLOOD PERFUSATES IN CAPILLARY CIRCULATION[1]

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Difficulties have been encountered in the perfusion of blood vessels with artificial solutions consisting of a mixture of salts as found in blood and a colloidal component to provide the necessary osmotic pressure. Unpredictable variations occur in the ability of such media to prevent the appearance of edema. Hitherto, most of the attempts to overcome this deficiency have been to increase the concentration of colloidal material or to change the nature of the colloid. Red cells have also been added to the artificial blood substitutes. The improvement noted was ascribed by Saslow (7) to the more adequate oxygenation provided by such solutions.

An investigation of this problem was undertaken by directly observing the capillary vessels in the frog through the microscope while the animal was being perfused. It was found, irrespective of whether the solutions were purely crystalloidal or contained colloidal components, that an active flow of the perfusate occurred only in the a-v capillaries, no circulation being apparent in the numerous true capillaries. Because of this, the use of solutions colored with Evans blue afforded a remarkably good demonstration of the a-v capillaries in contrast to the true capillaries. The existence of two types of capillaries in numerous tissues has been emphasized in a previous publication (9). An important distinction between the two is effected by the different way in which the vessels leave the parent trunk. The a-v capillaries are those vessels which are direct continuations of the arteriole and constitute a relatively small portion of the capillary bed. The a-v capillaries are of considerable length and are not to be confused with extremely short anastomoses between arteries and veins, which are often referred to as A-V-A vessels. Coming off almost at right angles from this continuous trunk are large numbers of true capillaries. The a-v capillaries, being direct extensions of the arterioles to the venules, serve as bridging channels between them. The numerous true capillary offshoots, by virtue of their position, do not lie in the direct path of flow of the perfusing solution.

[1] This study was made possible by a grant from the Josiah Macy Jr. Foundation.

Tests were made to ascertain the part played by the formed elements in the distribution of the perfusate. For this purpose, particulate matter, either as a suspension of carbon or of red cells, was added to the various perfusion media. The resulting capillary circulation, regardless of the type of particulate matter employed, was a decided improvement over the restricted circulation previously obtained. Within a short time the perfusate was seen to circulate through all of the capillary vessels and the appearance of edema was considerably delayed. Since the proper distribution of the perfusion fluid is of prime importance, the factors that enhance this feature were studied, with emphasis on the rôle of formed elements.

Zweifach (10) has found that marked differences occur in the capillary pattern of various tissues. Krogh (5), Saslow (7) and Drinker (3) have used the capillary vessels of the frog's web for perfusion studies. However, the capillary bed of the web belongs to the skin type of vessels and is atypical, consisting of a rich meshwork of extremely short anastomosing channels. The capillaries of the web are further unsuited since, as Krogh (6) and Conklin (2) have shown, they are abnormally permeable to proteins. The vessels of the mesentery and the tongue of the frog are more satisfactory for perfusion studies because their capillary pattern is representative of that found in connective tissues and muscle in general. Furthermore, the degree of permeability of the capillaries in the tongue and mesentery is directly comparable to that of similar vessels in other tissues.

For these reasons, the studies described in this paper were restricted to direct observations made on the capillary circulation in the mesentery and tongue of the frog while the animal was being perfused. Details of the perfusion technique employed are described in an earlier publication (1). The colloidal materials added to the Ringer solutions were 0.5 per cent ash-free gelatin (Eastman Kodak Co.) and 3 per cent gum acacia (E. Lilly). Evans blue, because of its poor diffusibility through the capillary membrane, has been used in blood volume studies by Gregersen and Gibson (4). Particle-free solutions were colored with Evans blue in order to follow the circulation of the perfusate more readily. The perfusion fluid was introduced by cannulating the aorta, the effluent fluid escaping through the cut end of the ventricle.

A. *Restricted circulation with solutions lacking formed elements.* Colored Ringer solutions buffered at pH 7.5 were employed first. When perfused by way of the aorta they readily appeared at the point of venous outflow. The perfusion solution was seen to enter the a-v capillaries immediately and washed out all the blood cells from these vessels. The perfusate did not enter the true capillaries.

Before starting the perfusion, a true capillary, that left the a-v capillary and rejoined it within the field of the low power objective, was selected for observation. As soon as the blood had been washed out of the a-v

capillaries, the blood cells inside the true capillaries were sucked into the a-v current from the two ends of the vessel. This continued until the true capillaries were completely emptied of blood cells. The suction-effect, by virtue of which material was continuously being removed from both ends of the true capillaries and draining into the a-v capillaries, seems to have arisen as a result of the rapid flow of the perfusate past the orifices in the a-v wall leading to the side capillaries. The disposition of the true capillaries, which come off almost at right angles to the parent trunk, favors the development of such suction forces, especially when a rapid flow is found only in the a-v capillaries.

A second series of Ringer solutions was prepared by adding mannitol, a relatively impermeable inert sugar, in an amount calculated to maintain proper osmotic balance. The circulation of this solution, likewise, was found to remain confined to the a-v capillaries.

A third series of media consisted of Ringer solutions combined with ash-free gelatin or with gum acacia. These perfusates were found to be more efficient in reaching almost all of the a-v capillaries. None of the colored perfusion fluid flowed through the more numerous true capillaries. When a higher concentration of colloidal material was used, the viscosity of the solutions (especially in cold blooded forms where the perfusate was kept at room temperature) severely slowed the flow and limited its distribution more radically than before.

When the Ringer-gelatin solution was perfused at a low pressure (15 to 20 mm. Hg), a flow existed only in a small number of a-v capillary channels. By keeping the rate of flow constant at this level, a steady state developed in which the true capillaries contained no active flow but remained open. These static true capillaries remained patent apparently as a result of fluid seeping into them from the tissues and being drawn at a slower rate into the a-v channels. When the pressure was increased, the streaming of the perfusate through the a-v vessels was accelerated. The true capillaries then gradually narrowed down over a period of 3 to 4 minutes and became completely collapsed along their mid-regions. The factor effecting this narrowing appeared to be the augmented suction-effect produced by the rapid flow through the a-v capillaries.

The true capillaries did not collapse during perfusions in which crystalloidal solutions were used, presumably because considerable fluid was diffusing into them from the surrounding tissues. An active flow was not observed in all the a-v capillaries, but only in those which took the most direct course to the venous vessels. This restricted circulation persisted even when the perfusion pressure was increased from the normal level of 30 mm. to as high as 75 mm. Hg.

These experiments showed that the beneficial action of colloidal matter resides in part in its ability to promote an active circulation of the per-

fusate through a much larger number of a-v capillaries than occurred with crystalloidal perfusates. The superior distribution of colloidal mixtures over that of mannitol-Ringer solutions, may be due to the fact that the viscosity of the colloidal perfusates more nearly approximates that of blood. In tissues where the a-v type of capillary predominates (tongue), such solutions were almost completely adequate in filling the capillary bed.

B. *Rôle of particulate matter in distribution.* A suspension of fine carbon particles or of avian red cells was added to Ringer-gelatin and Ringer-acacia solutions. When the animal was perfused with these solutions, the circulating fluid not only coursed through the a-v capillaries, but filled the entire capillary bed so that all parts of the tissue were simultaneously exposed to the action of the perfusate. The following is a description of the changes that occurred during the period in which the circulation of the perfusate became wider in scope to include the true capillaries.

When the particle-free colloidal solution had been washed out of the a-v capillaries by a carbon-containing perfusate, peculiar swirls of carbon were seen to develop at the orifices in the a-v wall leading into the true capillaries. These capillary offshoots are given off from the a-v parent trunk at a sharp angle and with a marked backward twist. Similar characteristic swirls appeared when suspensions of red cells were used. In addition, these were accompanied by the lodging of red cells against the angular projections of the wall at the point of capillary exit. The trapped cells wavered back and forth until the axial current was suddenly deflected into the side channels. The flow in the capillary side channels was sporadic in character, with the red cells being distorted and slowly forced through the narrowed portions of the vessels. The movement of this column of cells at first was pulsatile, the red cells being drawn back and forth through the constricted vessel. This was accompanied by a slow opening of that portion of the vessel with which the red cells made direct contact, until a continuous flow through the capillary was established. The number of side channels into which the flow was deflected varied with the perfusion pressure employed. At comparatively low pressures (15 to 20 mm. Hg), the flow through the true capillaries was sporadic. When the pressure was raised to 35 mm. Hg, the perfusate was carried into the majority of available channels.

This deflection of flow was repeatedly produced in the same preparation by the alternate use of particle-free and particle-containing solutions. At the commencement of perfusions with particle-free solutions, the flow became confined to the a-v channels as soon as the blood of the host had been washed out. Within 30 seconds after changing to a carbon containing solution, the perfusate gained access to all the capillary vessels. Carbon tended to stick to the capillary walls, especially along the intercellular cement, and often clogged some of the vessels. Red cells were never ob-

served to stick to the wall or to one another and were more efficient. For these experiments it was not necessary to use the cells of the host. Rooster red cells suspended in frog Ringer-gelatin solutions gave a perfectly normal picture, with the perfusate streaming through all of the capillaries.

From the above data it can be seen that the presence of the suspended material was responsible for the setting up of mechanical disturbances at the points along the a-v wall where capillary offshoots emanate and thereby routed the perfusate into all of the capillary channels.

C. *Particulate matter in the control of edema.* When particle-free Ringer or Ringer-mannitol solutions were used for perfusion, pronounced edema occurred within 10 to 15 minutes. Colloidal Ringer mixtures, lacking formed elements in suspension, were capable of maintaining normal fluid balance for 30 to 40 minutes. As previously indicated, colloidal perfusates maintain a more adequate capillary circulation. This probably is an important factor in the delayed edema obtained with colloidal solutions as compared with its rapid appearance with isosmotic Ringer-mannitol mixtures. Ringer-mannitol solutions left the a-v capillaries at an extremely rapid rate as compared to Ringer-gelatin mixtures. It is significant that the colloidal perfusates coated the inner surface of the capillaries and thereby not only rendered the wall relatively less permeable to fluid loss, but also curtailed the passage of the colloidal component of the perfusate into the tissues. Ringer-mannitol solutions do not exhibit such coating properties. The streaming of crystalloidal perfusate through the a-v capillaries was accompanied by a washing off of the coating of the blood proteins of the host, leaving the vessel wall in an excessively porous state. The effectiveness of both solutions, however, is considerably diminished because of their poor distribution, the perfusate coursing through only a small fraction of the available channels.

A suspension of carbon in Ringer-gelatin delayed the appearance of edema for over 110 minutes. Red cell suspensions were somewhat more efficient in this respect, delaying the onset of edema for more than 180 minutes.

The greater effectiveness of solutions containing particulate matter over those which are particle-free depends upon at least two important properties. First, the perfusates bring about a more normal type of circulation throughout all the vessels of the capillary bed. Secondly, the presence of the particles increases the oxygenation of the solution. Warburg (8) has shown that suspensions of carbon particles have considerably more oxygen than ordinary solutions because of the increased surface provided for the adsorption of oxygen. The presence of red cells also markedly increases the oxygen carrying capacity of the solution. These two factors acting together would tend to diminish anoxemic effects and insure a more adequate removal of metabolic products from all parts of the tissue.

Solutions lacking suspended material prevent edema only for a short period of time. The temporary lack of edema is in large part due to the limited filtration surface exposed to the perfusate, since only a fraction of the total capillary vessels are actually perfused by the circulating fluid. This situation results in general asphyxia and the accumulation of metabolites in most of the tissue. An increase in the permeability of the capillary wall follows, causing the onset of edema.

D. *Rôle of particulate matter in plugging.* In addition to its rôle in the distribution of the perfusate, particulate matter was found to perform another important function. Chambers and Zweifach (1) have shown that temporary porous spots appear in the capillary wall and can be increased or decreased by variations in pH and calcium content of the perfusate. In the present study, when the perfusion was carried out for longer than two hours, the capillaries became increasingly leaky. The increased outward diffusion of fluid was accompanied by a flattening of the particulate matter against the capillary wall over these points. This phenomenon, especially when red cells were used, acted to reinforce the weakened portions of the capillary wall and curtailed seepage through actual leaks by forcing the extremely plastic red cells into them. Stasis of red cells also played a part in preventing excess loss of fluid. The cells quickly filled the abnormally leaky vessels as the fluid diffused out of them. The resulting mass of cells clogged the vessel, removing it from the active circulation, and thereby functioned as an additional safeguard against excessive loss of fluid.

E. *Mechanism of collapse and closure of capillaries.* As has been noted above, the true capillaries frequently became narrowed, even to the extent of complete occlusion, in experiments with particle-free solutions where the circulation of the perfusate was limited to the a-v capillaries. An attempt is made here to compare these results with similar phenomena in animals with an intact blood circulation.

Perfusion data. The arrangement whereby the true capillaries come off almost at right angles to the a-v vessels exposes them to suction effects arising from the continuous flow in the a-v capillary. This was conclusively demonstrated when the blood of the host was washed out by perfusing the vessels with Ringer-gelatin solutions containing Evans blue. Despite the fact that none of the colored perfusate coursed through the true capillaries, the blood cells contained in them were rapidly drawn into the a-v current from both the arterial and venous ends of the true capillary. The blood cells moved in opposite directions towards both ends of the true capillary. Such a movement could only be brought about by a suction simultaneously acting on both the exit and entrance of the true capillaries.

During the perfusions with Ringer or Ringer-mannitol solutions, fluid was rapidly lost from the a-v capillaries, resulting in pronounced edema

within 10 minutes. In this case, the true capillaries, despite the absence of an active flow in them, always remained open. With Ringer-gelatin or Ringer-acacia perfusates, the loss of fluid was considerably diminished, as evidenced by the absence of edema during the first 30 to 40 minutes of perfusion. When the perfusion pressure was kept low (15 to 20 mm. Hg), the true capillaries remained patent throughout the experiment. However, a gradual collapse of the true capillaries occurred by raising the perfusion pressure to 35 mm. Hg. During this period, the a-v rate of flow was considerably increased and concurrently there was a corresponding diminution in the caliber of the true capillaries. The experimental findings suggest that the collapse of these vessels was a direct result of the increased suction forces created by the more rapid streaming through the a-v capillaries. The closure was first evident along the mid-regions of the true capillaries and progressively spread in both directions towards the arterial and venous ends of the vessel. Such capillaries remained closed throughout the remainder of the experiment despite the appearance of edema after 40 minutes of perfusion.

The differences in caliber of the true capillaries obtained with crystalloidal and colloidal perfusates, despite the complete absence of flow in these vessels, can be explained on the following basis. The degree of narrowing appears to be a resultant between the rate of infiltration of fluid into the true capillaries from the tissues and the removal of fluid from them by suction forces arising in the a-v capillaries. The rapid loss of fluid with crystalloidal perfusates provides an excess of tissue fluid. The true capillaries therefore are continuously being supplied with fluid seeping in from the tissues. In colloidal-Ringer perfusions, the rate of fluid loss is minimal during the initial 30 to 40 minutes. Fluid slowly diffuses into the static true capillaries for only a short time, no excess being available in the tissues. The continued suction then acts to effect a narrowing of the true capillaries, which is evident within 15 to 20 minutes.

It should be emphasized that no active closure of the true capillaries was observed. The capillaries appeared to be affected passively by the flow coursing through them and by the creation of suction forces in the a-v vessels. Opening of the true capillaries was never observed during the period when no flow circulated through them. Likewise, a collapse of the true capillaries occurred only when they were devoid of flow concurrent with the existence of an active circulation in the a-v capillaries.

The use of perfusates containing particulate matter brought about a complete reparation of this situation. When low perfusion pressures (15 to 20 mm. Hg) were used, the circulation was most active in the a-v capillaries and sporadic or non-existent in the true capillaries. By gradually increasing the pressure to 30 mm. Hg, an active streaming was obtained in all the capillary vessels. The initial effect was an increased rate of flow

through the a-v channels. A maximum rate of flow is reached, above which a further increase in pressure acted to distend the vessel. As the a-v capillary widens, the orifice leading into the capillary offshoots became more patent. The higher pressure in the a-v capillary also tended to produce a more rapid filtration of fluid into the tissues. When the perfusate contained red cells, the loss of fluid led to a closer packing of the red cells, especially on the venous side. The red cells were no longer solely confined to the central, axial a-v current but were dispersed throughout the vessel. Eddies appeared where the true capillary offshoots branched out and were effective in deflecting the circulation into these side channels. Carbon suspensions effected a partial opening of the true capillaries. Red cells, on the other hand, were forcibly squeezed into the collapsed channels and thereby distended them to a point where the cells coursed freely through the capillary.

Intact blood circulation. Similar observations to the above were made in tissues with an intact blood circulation. In resting tissues, a continuous flow exists only in the a-v vessels, the true capillaries remaining relatively static and often extremely narrowed. Following mild stroking of the tissue with a microneedle, the arteriolar flow to the capillaries in the area increases. The initial effect of this was a more intense flow through the a-v capillaries. Ten to fifteen seconds later, a closer packing of the circulating blood cells can be noted in the distal portions of the a-v capillaries and the flow began to slow down. This is evidently brought about in part by the added loss of fluid resulting from the increased pressure. The narrowed true capillaries can be seen to open slightly. Eddies appeared in the a-v capillary at the points where the side branches are given off and soon the flow was deflected into these capillaries. Following a variable period, depending upon the intensity of the irritation, the arteriolar flow diminished. The flow in the true capillaries became sporadic and gradually ceased. With the flow again limited to the a-v capillaries, conditions for the reëstablishment of the suction forces at both ends of the true capillaries are present. The contents of the vessel drain into the a-v capillaries and the true capillaries become gradually narrowed.

SUMMARY

Solutions designed to replace the blood as a perfusion medium to be completely effective must not only contain appropriate chemical constituents but also a suspension of formed elements, such as carbon or red cells. The beneficial action of the particulate matter lies, in part, in its ability to bring about a maximum distribution of the perfusate through all of the vessels of the capillary bed. In the absence of red cells or carbon, the flow is restricted to the a-v capillaries. The addition of suspended particles diverts the flow into the true capillaries which are offshoots of the a-v

vessels. Particulate matter, especially red cells, is effective also because it plugs up leaks in the walls of the vessels. Stasis in abnormally leaky vessels is an additional factor which acts to prevent further fluid loss by clogging these vessels and cutting them off from the active circulation.

I wish to thank Prof. Robert Chambers for his interest and suggestions throughout this work.

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THE PROLONGED ACTION OF ACIDIFIED SOLUTION OF PROTAMINE ZINC INSULIN

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In an earlier paper (1) from this laboratory, it was pointed out that when an acidified solution of insulin is subcutaneously administered it passes through various stages of hydrogen ion concentration until the reaction at the site of injection is in equilibrium with that of the tissue fluid, whereupon absorption takes place. On the basis of this theory, the question arises as to whether or not a clear aqueous solution of protamine zinc insulin would possess a prolonged hypoglycemic effect.

The prolongation of hypoglycemia following the administration of an insulin preparation to which protamine had been added in a phosphate buffered solution at about pH 7.3 was first reported by Hagedorn and his collaborators (2). Later Scott and Fisher (3) and Fisher and Scott (4) demonstrated that the addition of about 2 mgm. of zinc per 1000 units to such a protamine insulin preparation considerably prolonged its hypoglycemic effect. Since that time the addition of about 0.2 to 0.25 mgm. zinc per 100 units of protamine insulin has been a standard procedure.

This investigation is concerned with the preparation of a clear solution of protamine zinc insulin possessing a prolonged hypoglycemic action and a study of its effect on fasting rabbits.

GENERAL CONSIDERATIONS. Throughout this investigation a recrystallized preparation of insulin made in this laboratory was used. It assayed 22 international units per 1 mgm. The ash content did not exceed one per cent and its zinc content was about 0.3 mgm. per 1000 units.

Stock solution. A stock solution was prepared by dissolving 364 mgm. of insulin crystals in water to which had been added a few drops of one normal lactic acid. This solution was next transferred quantitatively to a 100 cc. volumetric flask and before diluting it to the 100 cc. mark 1.6 cc. glycerol and 0.1 cc. tricresol were added. The acidity of the solution was adjusted to about pH 3.0 (using the glass electrode). This stock solution contains 80 international units per 1 cc.

The following procedure is illustrative of the method employed for the

preparation of clear acidified solutions of protamine zinc insulin. Fifty cubic centimeters of the stock preparation containing 4000 units are transferred to an Erlenmeyer flask immersed in a water bath at a temperature of about 50°C. While stirring 200 mgm. of protamine are introduced followed by 7 mgm. of zinc as zinc chloride and 2 grams of sodium acid phosphate. The mixture becomes turbid at first but it clears up as the temperature reaches 45° to 50°C. The reaction of the mixture is adjusted exactly to pH 3.0 and the volume is increased to 100 cc. using a diluent containing distilled water, 1.6 per cent glycerol and 0.1 per cent tricresol. This mixture of an acidified solution of protamine zinc insulin consists of 5 mgm. of protamine, 0.2 mgm. zinc per 100 units of insulin in a 2 per cent phosphate buffer solution. Henceforth it shall be referred to as "clear solution of protamine zinc insulin."

In preparing dilutions for experimental work, the diluent unless otherwise specified consisted of a 2 per cent sodium acid phosphate containing glycerol and tricresol in the above mentioned ratios at an acidity of pH 3.0. In dilutions free from phosphate the addition of sodium acid phosphate was omitted.

Injections. All parenteral administrations were of deep subcutaneous injections. For dilutions containing 10 units per 1 cc. or less, a 1 cc. tuberculin syringe was used and the amount administered was proportional to bodyweight. However, when it became desirable to use a higher concentration of insulin such as 40 units per 1 cc. a micro syringe of 0.25 cc. capacity was employed.

Throughout this investigation the switch-over technique was used. The animals were divided into two equal groups. One group received one preparation and the other received another. The following week the order was reversed.

Rabbits that had been fasted for 24 hours were used. Blood was withdrawn from the marginal ear vein at the interval of time specified in each experiment. Sahyun's method (5) for blood sugar determination was employed.

EXPERIMENTAL. *1. The effect of dilutions.* In this experiment a comparison was made between the effects of each of the following preparations on the blood sugar of fasting rabbits:

A. A crystalline insulin preparation containing 5 units per 1 cc. *B*. A similar preparation of 5 units per 1 cc. of crystalline insulin to which had been added 2 mgm. zinc per 1000 units, and *C*, a solution containing 40 units per 1 cc. with 2 mgm. zinc per 1000 units. Twenty rabbits were used for each test and each animal received 2 units of insulin. The switch-over technique was employed. Samples of blood for sugar determination were withdrawn at 0, 1.5, 3, 4 and 5 hours respectively. The results of this experiment are found in figure 1.

2. Herein are shown the differences between the effects of *A*, a clear buffered solution of protamine zinc insulin; *B*, a precipitated protamine zinc insulin (the marketed preparation), and *C*, a control insulin preparation to which had been added a similar amount of zinc as in *A* and *B*.

Each preparation was tested on 20 rabbits. The concentration was 40 units per 1 cc. and each animal received 2 units of each preparation. The results of this experiment are summarized in figure 2.

3. The effect of aging on the clear solution of protamine zinc insulin preparation with reference to its hypoglycemic action was investigated on only one preparation. The tests were made when the preparation was 2, 3 and 4 days old. Each test was on 10 rabbits only. The switch-over technique was not employed. Each rabbit received 2 units of 40 units per

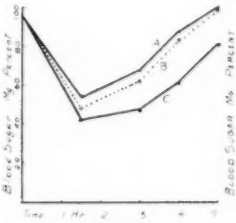

Fig. 1

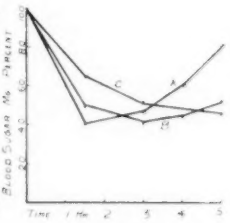

Fig. 2

Fig. 1. The blood sugar of fasting rabbits following the subcutaneous administration of 0.5 unit per kilogram bodyweight of: *A*, 5 units per 1 cc. dilution of crystalline insulin; *B*, 5 units per 1 cc. dilution of crystalline insulin containing 0.2 mgm. zinc per 100 units, and *C*, 40 units per 1 cc. dilution containing 0.2 mgm. zinc per 100 units of crystalline insulin. Each curve represents averages of 20 rabbits.

Fig. 2. The blood sugar of fasting rabbits following the subcutaneous injections of 2 units of 40 units per 1 cc. dilution of: *A*, solution of crystalline insulin; *B*, clear solution of protamine zinc insulin and *C*, protamine zinc insulin pH 7.2 (marketed product). Each curve represents averages of 20 rabbits.

1 cc. and blood samples were removed at 0, 1.5, 3, 5, 7 and 9 hours respectively. The averages of the data are shown in figure 3.

4. The effect of sodium acid phosphate was investigated in order to determine whether or not its addition had any influence on the hypoglycemic action of the clear solution of protamine zinc insulin. Two preparations were made with and without buffer, and each preparation was tested on 26 rabbits using the switch-over technique. A summary of the results is found in figure 4.

Discussion. It is sometimes difficult to demonstrate in the laboratory animal the prolongation of hypoglycemia following the parenteral administration of small doses of an insulin preparation endowed with prolonged activity. An example is the addition of 2 mgm. of zinc per 1000

units of insulin. Scott and Fisher (4) showed that for all intents and purposes the addition of such an amount of zinc to insulin does not appreciably enhance its action on rabbits, and in an earlier publication from this laboratory (1) their findings were confirmed. However, upon further investigation of this problem it was observed that the weak dilutions of this particular amount of insulin and zinc were responsible for the masking of its true action. It was also shown (1) that the addition of 2 mgm. of zinc per 1000 units of insulin in solution with the adjustment of the hydrogen ion concentration to about pH 7.0 caused the precipitation of about 75 per cent of the insulin protein, or preferably stated about neutrality 75 per cent of the insulin was adsorbed on the basic zinc salt. In view of this finding such an amount of zinc should appreciably prolong the action

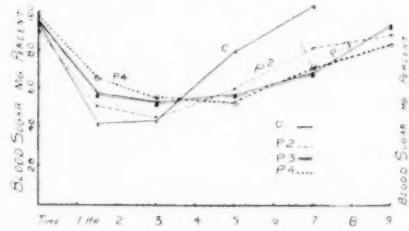

Fig. 3

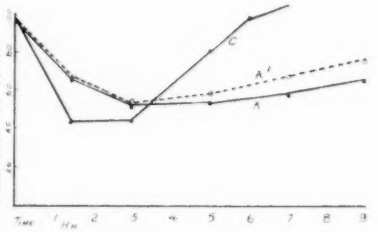

Fig. 4

Fig. 3. The blood sugar of fasting rabbits following the subcutaneous injection of 2 units of 40 units per 1 cc. dilution of clear solution of protamine zinc insulin. P-2—two days old, P-3—three days old and P-4—four days old preparations. Each curve represents the averages of 10 rabbits.

Fig. 4. The blood sugar of fasting rabbits following the subcutaneous injections of 2 units of 40 units per 1 cc. of buffered, *A*, and non-buffered, *A'*, clear solution of protamine zinc insulin. *C*, control crystalline insulin with zinc. Each curve represents the averages of 26 rabbits.

of insulin hypoglycemia in rabbits and some means should be discovered to make it demonstrable. Several experiments were performed that were of doubtful significance, until the concentration of the material to be injected was studied. Thus it became evident as shown in figure 1 that the administration of 2 units of insulin with zinc (2 mgm. per 1000 units) in concentration of 40 units per 1 cc. caused greater prolongation of hypoglycemia in rabbits than 2 units of the same preparation similarly given in concentration of 5 units per 1 cc.

Applying the well known physiologic principle of Donnan's theory of equilibrium to acidified solutions of insulin subcutaneously injected, neutralization at the site of injection must occur before absorption takes place. Basically, the principle involved in all preparations of insulin endowed with

prolongation of action is based on the decrease of their respective solubility at about the pH of the tissue fluid. The more insoluble a preparation is the greater its prolongation of action. Thus it was considered that an acidified mixture of protamine zinc insulin in a clear solution would cause a prolongation of hypoglycemia if the proper proportions of protamine to insulin were established. This was investigated in this laboratory and it was found that about 5 mgm. of protamine per 100 units of insulin with added zinc (0.2 mgm. per 100 units) yielded good results. The additions of 2.5 mgm., 10 mgm. and 20 mgm. of protamine to insulin were also studied, with somewhat similar results as shown in this paper. The various curves presented are self explanatory. *In vitro*, the amount of protamine necessary to cause the complete precipitation of the insulin protein is in the neighborhood of 0.75 mgm. to 1.25 mgm. per 100 units at about neutrality (4). *In vivo*, during the process of adjustment of ions of the clear solution of protamine zinc insulin at the site of injection the ratio of protamine to insulin should be greater since the size of the protamine molecule is considerably smaller than that of the insulin and undoubtedly a portion of the protamine not only would diffuse faster but there would be the possibility that it would combine with some of the tissue proteins. Consequently when equilibrium is reached it is necessary to have an adequate supply of free protamine to form the protamine zinc complex in the insoluble state at the site of injection.

Aside from the prolongation of hypoglycemia of the clear solution of protamine zinc insulin the data presented in figure 2 show the rapidity of the onset of its action as compared with the pre-precipitated protamine zinc insulin. This is clinically desirable as it would no longer be necessary to administer to the diabetic patient an adjunct dose of a fast acting insulin to take care of the blood sugar for the first few hours following the injection of protamine zinc insulin (pH 7.2).

The addition of phosphate to insulin preparations discussed in this paper seemed to enhance their action as shown in figure 4. However, the difference was not clear cut.

Unpublished data on the stability of the clear protamine zinc insulin in question are indeed favorable. Samples were incubated at 52°C. for 9 days with little loss in potency. Obviously this is very desirable.

SUMMARY

A method is described for the preparation of a clear acidified solution of protamine zinc insulin containing 5 mgm. of protamine and 0.2 mgm. zinc per 100 units. Its subcutaneous injection into fasting rabbits causes a prolonged hypoglycemia greater than that observed following the injection of a similar amount of crystalline insulin with and without added zinc.

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THE ACTION OF ALTERNATING CURRENTS UPON THE SPIKE-POTENTIAL MAGNITUDE, CONDUCTION VELOCITY AND POLARIZATION OF NERVE

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In previous communications from this laboratory (Rosenblueth and Reboul, 1939; Reboul and Rosenblueth, 1939) some effects of alternating currents (a.c.) upon nerve were reported. The present study deals with the changes produced by a.c. in the magnitude of the spike potential, the rate of conduction and the polarization of mammalian A fibers.

METHOD. The animals used were cats. They were anesthetized with dial (Ciba, 0.75 cc. per kgm.). The nerves studied were mainly the peroneal, occasionally the popliteal or the saphenous. They were dissected over as long an unbranched stretch as possible and placed in a moist chamber.

For studies of spike-potential magnitude or of conduction velocity the electrodes, both stimulating and recording, were silver or silver-silver chlorided wires. In the observations on changes of polarization the electrodes were calomel half-cells connected to the nerve through an agar-Ringer bridge.

Stimulation by single shocks was made with condenser discharges of various capacities and intensities. Repetitive stimulation was effected by condenser discharges through a thyratron, the frequency being controlled by a.c. These discharges were passed through a transformer before delivery to the nerve, in order to render them diphasic.

The sources of a.c. were two beat-frequency oscillators, a General Radio Company type 713B and a Clough Brengle Company model 79D. When the information was necessary a voltmeter indicated the output E.M.F., and a Weston thermocouple milliammeter, model 425, showed the intensity of current flowing through the nerve.

The spike potentials were led to a 5-stage capacity coupled amplifier and recorded from a cathode-ray oscillograph. When the recording electrodes were close to the region of the nerve where a.c. was applied, a capacity (0.01 to 0.1 μ F) was placed in parallel with the leads to the amplifier in order to reduce the a.c. artifact and the blocking effect of a.c. upon the amplifier. Although this procedure distorted the records, differences in magnitude or in delay were readily appreciated.

The changes of polarization were sometimes recorded on the cathode-ray oscillograph, after 3 to 5 stages of d.c. amplification. Tests of the electrodes and of possible thermal effects of large overloading a.c. voltages on the amplifier were made by substituting for the living nerve either the same nerve after killing it by immersion in hot water or a wick soaked in Ringer and repeating the experimental procedures used on the living nerve. A d'Arsonval galvanometer was also frequently used to record changes in polarization. Here again, tests made with dead nerves or with the living tissue, but after crushing between the a.c. and the lead-off electrodes, separated the genuine changes of polarization from possible spurious effects.

RESULTS. *Spike-potential magnitude.* Several precautions are necessary for the analysis of the changes of amplitude of the spike potential of a nerve trunk produced by applications of a.c. Thus, a decrease of the recorded spike-potential would result if some of the fibers failed to become active at the region of the recording electrodes, even when the spike potential of the active fibers could be full-sized or even increased in magnitude. Since a.c. may block the nerve impulses (Wedensky, 1903), a reduction in the number of active elements could in certain conditions be due to this block. A.c. may decrease the electrical excitability of nerve (Wedensky, 1903); test stimuli might therefore activate fewer elements during than before the passage of the current. Finally, as will be shown below, a.c. may decrease the conduction velocity of nerve impulses; an apparent reduction of the spike-potential magnitude could hence be merely a manifestation of temporal dispersion of the impulses corresponding to the different fibers in the nerve.

Two procedures were used to circumvent these sources of error. The first was to compare the magnitudes of the spike potential at various points beyond (with respect to the direction of the nerve impulses) the region where a.c. was applied. For convenience in describing the location of the several electrodes and their mutual distances a schema of the moist chamber is pictured in figure 1. If maximal test stimuli were delivered from a_1 to b_1 and a.c. was applied, for example, from c_1 to d_1, the responses recorded from any of the electrodes d_2, e_1 . . . . h_1 to the crushed region of the nerve at h_2 could only be due to fibers which had not been blocked by the a.c. Temporal dispersion would tend to decrease progressively the magnitude of such responses recorded in that order. If, then, the response at e_1 should be smaller than that at h_1 during the passage of a.c., it may be safely inferred that a.c. produces a decline of spike magnitude per fiber. This was readily found to be the case.

In figure 5 are illustrated results from a typical experiment. The lower records show the responses of the nerve at points 0.5, 1.5 and 3.5 cm., respectively, from one of the a.c. poles, during the passage of 15,000 cycles,

0.2 mam. The upper records show the control responses at the same points but without any a.c. It is apparent that the spike potential was more decreased in the neighborhood of the site of application of a.c. than in more distant regions of the nerve.

Figure 2 illustrates typical changes of spike magnitude at several points of a nerve, interpolar, polar and extrapolar, during the passage of a.c. of various intensities. It is clear that the depression is maximal at the a.c. poles, and becomes progressively less for more distant points. The magnitude of the response in the interpolar segment was always greater than at the poles; indeed, as shown in the figure, it was greater than at points at the same distance from one of the a.c. poles, but on the extrapolar region of the nerve. This apparent discrepancy is readily explained by the assumption that the passage of a.c. has not only a depressant effect upon the spike magnitude, but that it also has an independent increasing action, more visible in the interpolar segment than in neighboring extrapolar regions.

Although a significant increase of spike magnitude above the control level was never seen, the recognition that a.c. may cause such an increase explains both the changes in the interpolar segment and the complex shape of the curves in figure 2 for the extrapolar regions.

The second method employed to study changes in spike magnitude was as follows. The nerve was stimulated at one end and the recording electrodes were placed on intact regions in the middle of the excised trunk, so that a diphasic response was obtained. Applications of a.c. on either side of the recording electrodes should then result, if a.c. has a depressing effect more marked near the poles than in more distant regions, in a preferential decrease of one or the other of the two components of the diphasic record. This was found to take place (fig. 3).

With either of the two methods used for determining changes of spike-potential magnitude, the degree of the decrease varied proportionally with the intensity of a.c. applied. An influence of frequency was likewise recognizable. When different frequencies were applied with constant intensity lower frequencies (e.g., 200 to 2,000 cycles per sec.) had a greater depressing action than did higher frequencies (e.g., 2,000 to 15,000 per sec.).

Changes in conduction velocity. Certain difficulties have again to be considered for the interpretation of records showing an apparent slowing of the nerve responses during or after applications of a.c. Thus, if the fastest fibers in the nerve were more readily blocked than elements of slower conduction rate, a comparison of the measurements of conduction velocity taken before and during the passage of the current might not be legitimate, for such measurements could apply to different elements in the nerve.

That a.c. can slow considerably the conduction rate is clear, however,

from the following data. The responses illustrated in figures 3, 4 and 7 show not only a more delayed beginning during or after than before a.c., but also a delayed subsidence. The latter delay may be safely interpreted

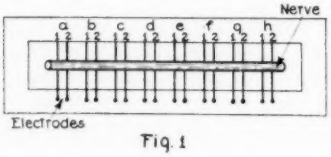

Fig. 1

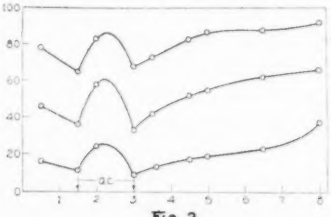

Fig. 2

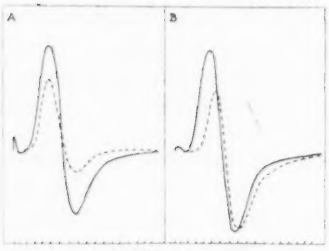

Fig. 3

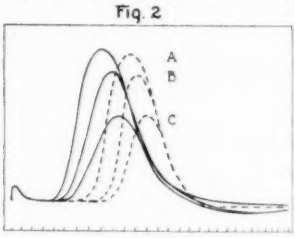

Fig. 4

Fig. 1. Schema of the moist chamber. The electrodes are arranged in pairs. The distance between the members of each pair (i.e., from a_1 to a_2; from b_1 to b_2; etc.) is 0.5 cm. The distance between neighboring pairs (i.e., from a_2 to b_1; from b_2 to c_1; etc.) is 1 cm.

Fig. 2. Changes of spike magnitude during the passage of a.c. (frequency 15,000 cycles per sec.). Ordinates: spike magnitude during the application of a.c. expressed as per cent of the responses obtained at the corresponding points before a.c. Abscissae: centimeters. The stimulating cathode was at O; the arrows indicate the points in the nerve at which the a.c. was delivered. Average voltage of a.c.: A, 1 v.; B, 2.2 v.; C, 3.2 v.

Fig. 3. Changes of spike magnitude during the passage of a.c. (frequency 15,000 cycles per sec.). Stimulating electrodes at a_1 and b_1 (see fig. 1). Diphasic records from d_2 to e_2. The records were drawn by projecting the original film through a photographic enlarger. Solid lines: control responses without any a.c. Broken lines: responses during delivery of a.c. : A, a.c. (2 v.) applied from c_2 to f_2. B, a.c. (1.5 v.) applied from c_2 to d_2. Time scale: 0.2 msec.

Fig. 4. Changes of spike magnitude and of conduction velocity during the passage of a.c. of progressively increasing intensity (A, B, C, solid lines) and progressively decreasing intensity (C, B, A, broken lines). The records were drawn by projecting the original film through a photographic enlarger. Time scale: 0.2 msec.

as indicating a slowing of some of the originally slow elements in the nerve, dismissing as unlikely the alternative possibility that the time course of the spike potential per fiber should be greatly modified by a.c.

Measurements of the conduction rate for different segments (e.g.,

1.5 to 3.0 cm.) of the interpolar and distal extrapolar region of the nerve clearly indicate, likewise, that a marked slowing may take place. The conduction rate of the fastest elements responding beyond the site of application of a.c. increases progressively with the distance from the a.c. electrodes. Thus in a typical experiment the stimulating electrodes were placed at a_1 and b_1, and the a.c. electrodes at c_2 and c_1. The conduction velocity measured for different segments of 2.5 cm. along the nerve was uniformly 70 m. per sec. before a.c. was applied. During the application of 15,000 cycles per sec. (5 v.) the conduction velocities measured for the

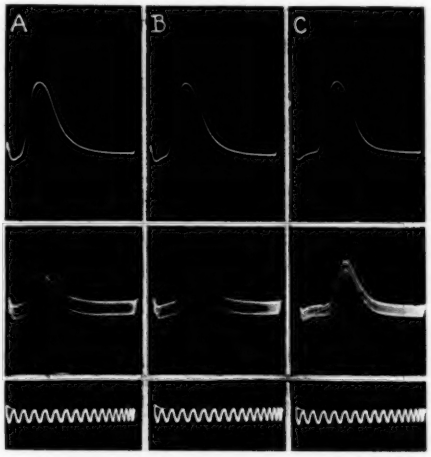

Fig. 5. Changes of spike magnitude during the passage of a.c. Stimulating electrodes at a_1 and b_2. A.c. electrodes d_1 and c_1. Records: A from e_2, B from f_1 and C from g_2, to the crushed end at h_2. Upper records: controls without a.c. Lower records: during the passage of a.c. (frequency 15,000, 1.2 v.). Time signal: 2,000 cycles.

segments from c_2 to c_1 (interpolar segment), from d_2 to f_1, and from e_2 to g_1 were approximately 45, 50 and 60 m. per sec., respectively.

As was the case for changes of spike magnitude, the degree of slowing produced by a.c. was greater for more intense than for weaker currents. With constant intensity relatively low frequencies were more effective than higher frequencies.

Changes of polarization. Applications of a.c. may change markedly the resting polarization of nerves. The changes at or near the a.c. poles are detectable with reference to more distal regions of the nerve or as changes of the demarcation potential. The results were complex. Relative positivity or relative negativity of the region treated by a.c. or of neighboring segments could be recorded.

In general, the sign and magnitude of the polarization change depended on the intensity and frequency of the a.c. applied and on the distance of the region of the nerve investigated from the site of application of a.c., as follows. When, with constant frequency, the intensity of the a.c. applied was progressively increased, the regions of the nerve at the poles or in the close neighborhood first became progressively more negative with respect to a distant crushed point (cf. Bishop, 1932). Further intensification resulted, however, in decreased but more prolonged negativity,

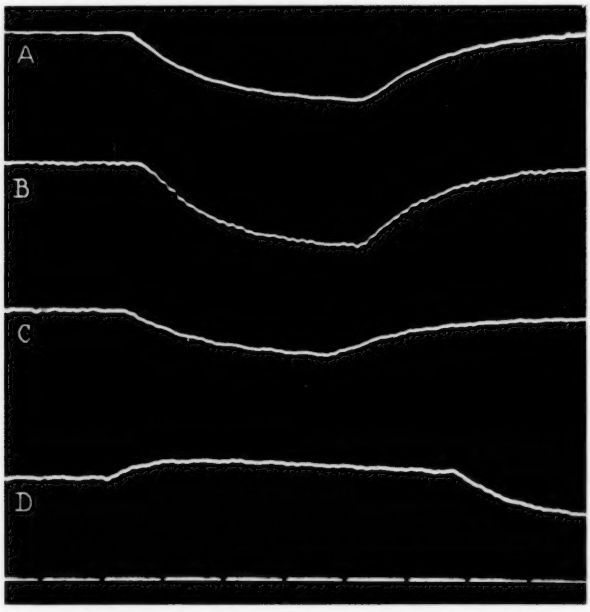

Fig. 6. Changes of polarization. A.c. electrodes at a_1 and c_1. Leads to the galvanometer from c_2 and e_1. Downward excursions denote negativity of c_2 with respect to e_1. The a.c. applied was in all cases with a frequency of 200 cycles per sec. The voltages were A, 1; B, 3; C, 7; and D, 17. Time scale: 1 sec. Crushing the nerve between c_1 and c_2 resulted in the disappearance of all the changes recorded.

as compared to the effects of weaker currents (fig. 6). Stronger intensities led usually to positivity during the passage of the current, followed by prolonged negativity after cessation of the a.c. (fig. 6D, see Wedensky, 1903).

The influence of the frequency of the a.c. was readily seen by the application of different frequencies with a constant intensity. The effects with relatively high frequencies (e.g., 15,000 cycles per sec.) were much smaller than with slow frequencies (e.g., 200 per sec.). If, however, the

high frequencies were made sufficiently intense the results were similar to those obtained with weaker, slower frequencies.

The distance from the a.c. poles was likewise an important factor in determining the sign and magnitude of the changes of polarization observed. Thus, when a strong current was applied, so that the region of the nerve at the poles or in the close vicinity became relatively electropositive, regions slightly more distant from the poles could register electronegativity with respect to a far, crushed reference point.

The independence of the several effects of a.c. upon nerve. It was interesting to attempt to correlate the effects of a.c. upon the different aspects of nerve function studied here with each other and with the effects of electrical excitability described previously (Reboul and Rosenblueth, 1939). It was also interesting to investigate whether or not the effects of a.c. could be correlated with the well-known electrotonic actions of d.c. A specific question which arises in analyzing such a possible correlation is the following. Is decreased polarization always associated with decreased spike magnitude and increased electrical excitability and conduction velocity, as the data on electrotonus and the membrane theory suggest? Several experiments were planned to answer this question.

That the changes of spike magnitude may be independent of the changes of conduction velocity is indicated by the following observations. In figure 4 are illustrated typical effects upon the nerve responses when a.c. was applied continuously for a prolonged period, first with progressively increasing and then with progressively decreasing intensity. The stimulating electrodes were placed at a_1 and b_1 (fig. 1); a.c. was delivered from d_1 to f_1 and the record was taken from g_1 to h_1. The solid lines show the responses during the application of progressively more intense a.c., and the dotted lines the responses when the a.c. was gradually weakened. Although the record was not taken at one of the a.c. poles, where the slowing was probably maximal, the discrepancy between the effects on spike magnitude and on conduction velocity, respectively, in the responses during progressive intensification as compared with the responses during gradual weakening of the a.c., clearly indicates the independence of the two depressive actions.

A similar discrepancy between the recovery of spike magnitude and that of conduction velocity is illustrated by a different method in figure 7. The stimulating electrodes were at a_1 and b_1; the a.c. electrodes from c_1 to g_1; and the records were taken from g_1 to h_1, that is, from the region at one of the a.c. poles to the crushed distal end. The large rapid response is the control before application of a.c. The progressively increasing slower spikes were photographed at about 3-sec. intervals after a.c. (5,000 cycles per sec.; 0.6 mam.) had been applied for 5 sec.—i.e., during recovery from complete block.

The facts which indicate that spike magnitude and conduction velocity may vary independently may be summarized by the statements that as a rule weak currents can have striking action on spike magnitude with only slight effects upon conduction velocity, and after prolonged or strong applications of a.c., the slowing of conduction can long outlast the depression of spike magnitude.

That the electrical excitability of nerve can vary independently of the changes in spike magnitude and in conduction velocity is shown by the following observations. Reboul and Rosenblueth (1939) found that the electrical excitability of the extrapolar regions of the nerve near the a.c.

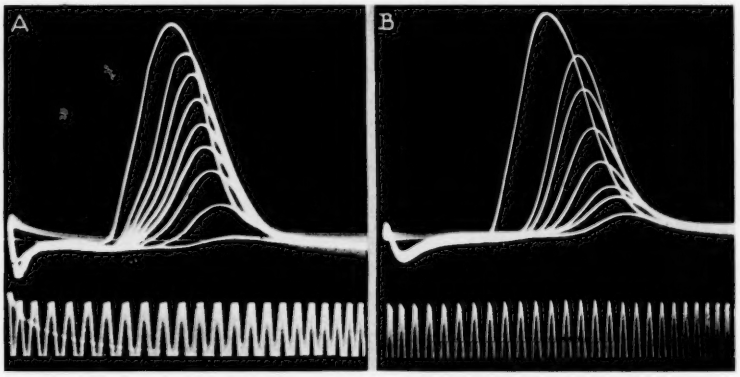

Fig. 7. Independence of the recovery of spike magnitude and of conduction velocity after a strong application of a.c. Stimulating electrodes at a_1 and b_1. A.c. electrodes at c_2 and g_2 for A, and at d_2 and f_2 for B. Records from g_2 and f_2 for A and B, respectively—i.e., from the region of the a.c. pole—, to the crushed region at h_2. The responses were photographed without moving the camera. The largest and quickest control response was first taken. A.c. (frequency 5,000 cycles per sec., intensity 0.6 mam.) was then applied for 5 sec. Further pictures were then taken at about 3-sec. intervals as the responses, which had been entirely cancelled by the a.c., progressively grew back toward normal. Time calibrations: 5,000 cycles.

electrodes decreased when weak a.c. was applied and increased upon application of strong a.c. The present study, on the other hand, shows that the spike magnitude at those regions decreases progressively as the a.c. is intensified. This absence of correlation was tested on a given nerve as follows. Stimulating electrodes were placed at a_1 and b_1 and a.c. electrodes at c_1 and d_1. The maximal spike magnitude was recorded at c_1, by leading from that point to the crushed region at h_2, before and during the passage of a.c. with a fixed frequency and various intensities. The changes of electrical excitability at c_1 were then measured by sending submaximal condenser discharges of constant capacity and amplitude

from d_2 to c_1 (cathode) and recording the responses from g_2 to h_2 before and during the passage of a.c. with the same frequency and intensities that had been used before. The results were as usual; the spike magnitude progressively decreased as the a.c. was intensified, while the electrical excitability first decreased and then increased at the point tested.

The data on changes of polarization (fig. 6) clearly indicate the independence of these changes from those of spike magnitude and conduction velocity. As a.c. of a given frequency is progressively intensified spike magnitude and conduction velocity gradually decline, while relative

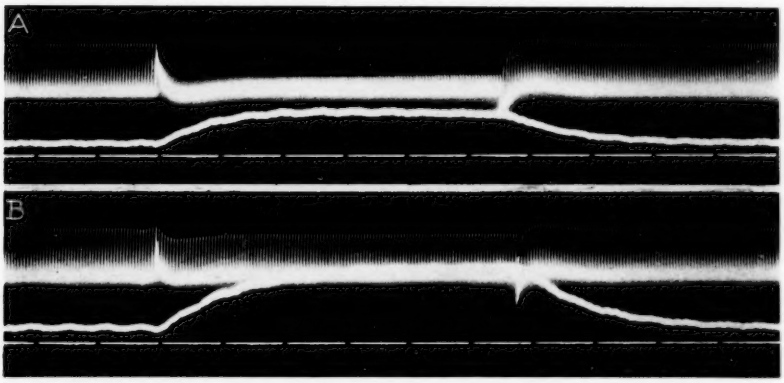

Fig. 8. Changes in spike magnitude and in polarization produced by a.c. and by d.c. (catelectrotonus). Stimulating electrodes at a_1 and b_1. A.c. or d.c. electrodes (impolarizable) at d_1 and c_1. Recording electrodes (impolarizable) at e_2 and f_2. The leads from these electrodes were made in parallel to the galvanometer and the capacity-coupled amplifier. Two additional capacities (0.1 μ F) were placed in each of the leads to the amplifier to prevent any d.c. interference from this source to the galvanometer. Upper tracing: nerve spike-potentials recorded from the cathode-ray oscillograph. Lower tracing: galvanometer; upward excursions denote relative negativity of d_1 with respect to c_1. Time: 1 sec.

A. A.c., frequency 10,000, average amplitude 9 v.

B. D.c., 0.5 v.

negativity first increases and later decreases to be substituted in some cases by relative positivity. This independence is also emphasized by the comparison of the effects of a.c. with those of d.c. In a series of observations, with stimulating electrodes at a_1 and b_1, a.c. or d.c. was applied from c_2 to c_2 and the responses were recorded monophasically from f_1 to h_1. Typical results are illustrated in figure 8. Invariably when the a.c. and the d.c. were adjusted so that approximately equal changes of polarization ensued the spike magnitude was more and longer depressed by a.c. than by d.c.

A comparison of changes of polarization with changes of electrical excitability again reveals absence of correlation. Throughout the range of intensities of a.c. which result first in a decrease and then in an increase of electrical excitability the region of the nerve tested may show relative negativity with respect to a distant reference point.

Discussion. There is no reason to assume that a.c. will have any effects on nerve qualitatively different from those produced by d.c. It would be expected, therefore, that the action of a.c. should be explainable as an interaction of anelectrotonus and catelectrotonus.

Prolonged applications of a.c. have more marked effects than brief applications. It is obvious, therefore, that the anelectrotonic and catelectrotonic effects corresponding to each half-cycle, respectively, do not cancel their effects. The conclusion is supported by the fact that the after-effects of a.c. of a given frequency and intensity are consistent—i.e., they do not depend on the sign and amplitude of the current at the time when it is stopped.

If anelectrotonus and catelectrotonus correspond to actions of the current of opposite sign on the same (one or several) structures or processes in nerve a lack of cancellation would take place if the cathodal and anodal effects are asymmetric in their amplitude, their spatial distribution or their time course. Such asymmetry was established by Pflüger (1859) and has been abundantly confirmed. With weak d.c. the cathodal effects are more prominent and widespread than the anodal effects. Strong currents lead to the opposite result. It would be expected accordingly that weak a.c. might be similar in action to catelectrotonus, while strong a.c. would duplicate the results of anodal polarization. In table 1 are summarized some effects of weak or strong a.c. or d.c. It is apparent that the expectation mentioned is not confirmed.

Two alternative views are compatible with the data. The cathodal and anodal effects may not be due to opposite actions on the same processes or structures but might be caused by influences exerted at different steps in the nerve. In such a case they could co-exist independently without mutual cancellation. The second suggestion is that the cathodal and anodal actions are not simple but complex. Thus, during weak catelectrotonus there might be not only an influence increasing the electrical excitability but also a marked tendency to decrease this excitability. This view could account for the reversal of effects which commonly takes place upon cessation of the d.c. applied, a reversal which has not received thus far an adequate explanation. There is no evidence available to support or reject either of these two hypotheses.

The discussion has dealt thus far with the effects of the electric current upon nerve. It is now interesting to consider the bearing of the data on the problem of the interrelation of different aspects of nerve function.

The data illustrated in figures 4, 7 and 8, and those summarized in table 1 emphasize the large degree of independence of the several features of nerve function studied. Conduction velocity depends probably on at least two factors, the amplitude of the stimulus delivered by the active region and the excitability of the succeeding segments. It is not difficult, therefore, to conceive that it might vary independently of the changes in either of these two factors considered singly.

The membrane theory suggests, on the other hand, that the spike amplitude should vary directly, and the electrical excitability inversely as the demarcation potential. The present data do not support this suggestion. Other facts can be quoted in support of the independence of the variables in question. Thus, as shown by Bishop and Erlanger (1926), a rise of temperature increases the demarcation potential and may increase the spike magnitude, but it results in an increase, instead of a decrease, of electrical excitability. Similarly, Bishop (1932) found that cocaine, which

TABLE 1

A comparison of some actions of a.c. with the corresponding effects of d.c.

The signs indicate: +, increase; 0, no change; −, decrease

	DEMARCATION POTENTIAL	SPIKE MAGNITUDE	ELECTRICAL EXCITABILITY	CONDUCTION VELOCITY
Weak a.c.	−	−	−	−
Strong a.c.	0, +	− −	+	− −
Weak catelectrotonus	−	−	+	+
Strong catelectrotonus	− −	− −	−	−
Weak anelectrotonus	+	+	−	−

produces no significant change in the amplitude of the spike potential, increases slightly the demarcation potential and reduces markedly the electrical excitability.

A theory of nerve conduction that covers the data is not available. For the present the suggestion which emerges is that if the three variables discussed are functionally related, as there is good reason to believe, the expressions for this relationship should include at least two independently variable parameters, to allow for the number of degrees of freedom in the system. Thus, if the development of the spike potential corresponds not to a total depolarization of an interphase but to a partial breakdown, capable of independent variation, the observed independence between the demarcation potential and the amplitude of the spike could be readily accounted for. Similarly, if the electrical excitability should be interpreted as depending not exclusively on the dgree of initial polarization but also on some additional independent factor, such as the rate of movement of

the ions concerned, then the independent variations of the demarcation potential and the electrical excitability would be explained.

SUMMARY

Applications of alternating currents (a.c.) to nerves result in a decrease of the amplitude of the spike potential at and in the neighborhood of the a.c. poles (figs. 2, 3 and 5).

The conduction velocity of nerve is slowed by a.c. (figs. 3, 4 and 7).

The demarcation potential is either decreased or, less commonly, increased by a.c. (figs. 6 and 8).

These several changes and those of electrical excitability (Reboul and Rosenblueth, 1939) may occur independently (figs. 4, 7 and 8; table 1).

The data are discussed from the standpoint of the problem of the actions of electric currents on nerves and from that of the problem of the functional relationship between the demarcation potential, the spike-potential amplitude and the electrical excitability of nerve.

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DECREASE OF HISTAMINASE IN TISSUE BY ADRENALECTOMY AND ITS RESTORATION BY CORTICO-ADRENAL EXTRACT

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In a previous communication a method for the determination of histaminase in the tissues of the rat was given (Rose, Karady and Browne, 1940). It was shown that the only tissues containing the enzyme in this species are lung and intestine. It is well known that the resistance of the rat and other species to histamine is markedly diminished following adrenalectomy (Gottesman and Gottesman, 1928) (Rose and Browne, 1938). It has further been shown that the administration of cortin to an adrenalectomized rat will restore the resistance of this animal to histamine to normal (Gottesman and Perla, 1931) and the ability to inactivate histamine (Rose, 1939).

In the following experiments, the effect of adrenalectomy on the histaminase content of rat lung was studied. A preliminary report of this has already been given (Rose and Karady, 1939). Further observations on the effect of treatment with cortin on the histaminase content of the lung of the adrenalectomized animal are reported in the present communication.

METHODS. Rats both male and female of a hooded strain weighing from 140 to 180 grams were used. Under ether anesthesia, bilateral adrenalectomy was performed and after allowing the animals to recover, they were placed on a purina diet and given normal saline to drink. After an interval of 7 to 10 days, the time at which the diminished resistance to histamine is known to be well established (Rose and Browne, 1938) the animals were again anesthetized with ether, and observing sterile precautions, the lungs were removed, washed free of blood in normal saline, and then placed on large sheets of filter paper to remove the excess moisture. At the same time, lung tissue from intact animals was removed in a similar manner. The lungs of all the animals in each group were then ground up, mixed, and an accurately weighed amount, usually 0.5 gram, placed in Erlenmeyer flasks of 75 cc. capacity containing 20 cc. of phosphate buffer of pH 7.2. To this was added 1 cc. of a histamine HCl solution

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containing 1000 γ of histamine base per cubic centimeter. After the addition of 10 drops of Toluol, the flasks were placed in an incubator at 38°C. for 16 hours. They were then removed, heated to 90°C. for five minutes and then made up to 50 cc. final volume. The histamine content was determined by the guinea-pig-ileum method.

RESULTS. a. *Effect of adrenalectomy.* Five groups of 5 animals were used in this series. The first three were maintained on saline for seven days, and then used. The results are shown in table 1. The figures

TABLE 1

The effect of adrenalectomy on the ability of rat lung tissue to inactivate histamine

EXPERIMENT NUMBER	CONTROLS		ADRENALECTOMIZED	
	Weight of tissue	Amount histamine destroyed	Weight of tissue	Amount histamine destroyed
			Maintained on normal saline for 7 days	
	grams	γ	*grams*	γ
157	0.5	800	0.5	600
	0.5	780	0.5	600
			0.5	600
158	0.4	750	0.6	750
	0.4	750	0.4	650
	0.2	400	0.2	300
159	0.5	800	0.6	700
	0.5	750	0.5	700
			0.2	300
			Maintained on saline for 7 days and on water for 3 additional days	
164	0.5	800	0.5	400
	0.5	700	0.5	400
			0.5	300
167	0.5	800	0.5	400
	0.5	700	0.5	400
			0.5	300

Tissue incubated with 1000γ histamine for 16 hours.

given denote the amount of histamine destroyed in 16 hours by varying weights of tissue. It will be observed that in experiments 157, 158, 159 only a moderate decrease in the histaminase content occurred (approximately 20 per cent). Since it is known that adrenalectomized rats may be maintained for long periods of time on normal saline alone, it was decided to place the animals on water for three additional days. Such animals become quite weak and die within 4 to 5 days. The results of this procedure on the histaminase content of lung are shown in experiments 164 and 167.

table 1, and experiments 170, 182 and 188, table 2. It will be noted that there is a marked diminution in the histaminase content of the tissue, 40 per cent of the histamine being destroyed as compared to 75 per cent in the same time by an equal weight of tissue from intact animals.

b. *Effect of administration of cortin.* Six groups of adrenalectomized animals were used. They were maintained on normal saline for seven days. Three of these were then given 2 cc. of Wilson's Cortin[2] intramuscularly twice daily for 4 days and the saline replaced by water. On the 12th day the animals were killed and the tissues removed in the usual manner. The other three groups were taken off saline on the 7th day and

TABLE 2

The effect of the administration of cortin to adrenalectomized rats on the ability of lung tissue to inactivate histamine

EXPERIMENT NUMBER	HISTAMINE DESTROYED BY 0.5 GRAM OF LUNG TISSUE		
	Controls	Adrenalectomized, maintained on normal saline 7 days and on water for 4 days	Adrenalectomized, maintained on normal saline 7 days and on cortin for 4 subsequent days plus water to drink
	γ	γ	γ
170	750	400	600
		450	500
		300	700
			750
			700
182	700	400	600
		500	600
		300	600
			600
188	650	500	700
		525	700
		500	700
			700

Tissue incubated with 1000γ histamine for 16 hours.

placed on water for the remaining four days, at which time they were used. There were also three groups of intact animals whose lung tissue was removed and incubated in the same manner. The results are shown in table 2.

It will be observed that the ability of the lung tissue of adrenalectomized animals to destroy histamine is restored by the administration of cortin in the amounts indicated, so that an average of 65 per cent of the added histamine is inactivated by 0.5 gram tissue.

[2] Supplied through the courtesy of Dr. D. Klein and the Wilson Laboratories, Chicago.

DISCUSSION. The decrease in resistance to histamine following adrenalectomy may be explained in part by a decrease in the histaminase content of tissue, based upon the above results. Since in the rat, the major part of histamine inactivation takes place by the kidney and liver (Rose and Browne, 1938), and since neither of these two organs contains histaminase in this species (Rose, Karady and Browne, 1940) it does not seem likely that the decrease in histaminase is a major factor in causing the delay in the destruction of injected histamine in the adrenalectomized animal. It may, however, play a minor rôle. Furthermore, the adrenalectomized rat maintained on normal saline is markedly sensitive to histamine as compared to the normal intact animal and, as noted above, there is only a moderate diminution in the histaminase content of the tissue, unless the adrenalectomized animals are deprived of normal saline. It is also interesting to note in this connection that there is an increase in the histamine content of the small intestine and stomach of adrenalectomized rats, and that this increase is even greater when the animals are given water instead of normal saline to drink (Rose, 1940).

It has been shown that the ability of the adrenalectomized rat to destroy histamine may be restored to normal by the administration of adequate amounts of cortin (Rose, 1939). This may also be in part accounted for by the above results. It is known that anaphylactic shock is much more readily produced in the adrenalectomized rat than in the intact animal (Wyman, 1929). This might be explained in part by the fact that there is a decrease in histaminase and an increase in the available histamine in certain tissues.

CONCLUSIONS

The effect of adrenalectomy on the histaminase content of the tissue of the rat has been studied. It has been shown that if animals are adrenalectomized, maintained on a standard diet, and given normal saline to drink, there occurs a diminution of the histaminase content of the lung. If water is substituted for the normal saline, an even greater diminution occurs. The diminution of histaminase in the lung tissue of the adrenalectomized rat may be restored to within normal limits by the administration of adequate amounts of cortin. The significance of these findings is discussed.

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OPACITIES OF THE LENS INDUCED BY ADRENALIN IN THE MOUSE

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Rapidly precipitated transitory opacities of the lens have been noted in laboratory animals incidental to the following conditions: slow asphyxia —rat (1); "alarm reaction"—rat, cat (2); toxic substances (especially adrenalin)—rat, mouse (3). Extensive studies on the more slowly developed cataracts of metabolic origin have been published (galactose feeding, naphthalene, deficiencies in the components of the vitamin B_2 complex—cf. 4) but the acute reversible type has in all probability been passed over by many observers as toxic cataract of little significance in the physiology and pathology of the human lens. Biozzi (1) studied it in some detail from the physiological and histological standpoint. Nordmann (6, 7) in a résumé of his researches on cataract discussed it at length. In this and in the more recent review (5) are mentioned Collevati's observations on the regular occurrence of opacities following the administration of adrenalin, which apparently still lack confirmation.

Recently the 1909 report of Schultz (3) on the comparative activity of various adrenalin preparations has come to my attention, in which he describes in detail this toxic effect of adrenalin for the mouse lens. Although the frequency of its occurrence was not explicitly stated, his observations on the effective range of adrenalin dosage, the speed of onset, the complete reversibility of the cataract, and the unpredictable variation in the reaction of the mouse were in close agreement with the observations reported below.

METHODS. Originally the limited data available suggested that the change in the lens might be associated with a combination of adrenalemia and shock. Therefore the effects of single toxic intraperitoneal injections of adrenalin alone (adrenalin chloride, 1:1000 sol., Parke Davis), of histamine alone (ergamine acid phosphate, Burroughs Wellcome), or of histamine followed by adrenalin, were observed as regards changes in the lens in male and female mice (average weight 20 grams).

RESULTS. *Adrenalin.* Within 3 to 10 minutes from the time of injection of from 0.3 to 0.7 mgm. per 100 grams of the drug, the following train

of symptoms occurred: quietly increasing prostration, dyspnea, lachrymation, salivation, cyanosis, exophthalmos, and in severe states reduction or absence of the light reflex. Individual variation was marked in some animals (cf. 3), the severity of the reaction with the smallest amount of adrenalin in some being equal to that with the maximum dose in others. The degree and frequency of the response tended to be increased after moderate fasting (4–6 hrs.), definite rigor appearing in a few. The total mortality for non-fasted mice averaged but 11.8 per cent in this range of dosages but suddenly increased to 70 to 100 per cent starting at 0.8 mgm. per 100 grams with an occasional mouse surviving 1.0 mgm., while the total mortality of fasted mice reached 19.9 per cent because of the increased number of delayed deaths, i.e., incomplete recovery and relapse (shock-like death).

Single or double transitory opacities developed within the first hour after the injection, with increasing frequency (from 10 per cent to 57 per cent) as the dosage of adrenalin increased from 0.3 mgm. to 0.7 mgm. The average time of onset was 30 minutes, although changes in the lens were often visible under magnification in 15 minutes. In a few, 60 minutes elapsed before the development of an initial diffuse cloud which rapidly progressed in intensity with marked precipitation of the suture pattern. The depth of the opacity varied with the individual from an irregular small area of clouding with a partially defined suture pattern, to a heavy uniform cloud with a dense "Y" formation, or the solid white marble eye noted by others. The duration of the cataract was proportional to the density attained, a light opacity reaching maximum and clearing within 30 minutes, whereas an intense cataract required about one hour to reach maximum and two to three hours for dispersion. The first signs of clearing started with a more or less central loss (breaking) of the uniform turbidity with a slower fading of the arms of the "Y". In vivo and in vitro the whitish substance seemed to recede toward the equator of the lens.

No consistent correlation was apparent between the intensity of the cataract and the degree of collapse, exophthalmos, cyanosis, etc., an occasional positive case retaining an active light reflex.

Biopsies of eyes showing varying degrees of opacities confirmed the gross observations that the reaction was limited strictly to the lens. The clouding was due to the formation of a subcapsular semifluid milky substance confined to the superficial cells of the anterior cortex. According to Biozzi (1) no histological changes are found in the epithelium or the capsule other than an occasional vacuolization of the superficial cortical fibres. Since examination of a typical specimen of an adrenalin-induced opacity confirmed this, further histological studies were postponed.

Histamine. As may be seen from the table, the mice reared in this laboratory showed the usual high resistance to histamine, 3 mice surviving

180 mgm. per 100 grams. There were no visible alterations in the clarity of the lens following injection.

Histamine and adrenalin. The injection of adrenalin (0.5 and 1.0 mgm. per 100 grams) was given 10 to 20 minutes after histamine before the characteristic symptoms of shock had appeared (circulatory stasis, cyanosis, exophthalmos).

TABLE 1

DESCRIPTION	HISTAMINE PER 100 GRAMS	ADRENALIN PER 100 GRAMS	NUMBER USED	APPEARANCE OF OPACITIES			DISAPPEARANCE OF OPACITIES		MORTALITY, < 1 HOUR		MORTALITY, ± 12 HOURS		SURVIVAL	
				Number	Per cent	Time	Number	Time	Number	Per cent	Number	Per cent	Actual number	Possible number
	mgm.	*mgm.*				*minutes*		*minutes*						
Normal		0.3	11	1	9.9	15-20	1	45	0	0	0	0	11	0
Normal		0.5	84	36	42.7	15-20	20	60-150	8	9.5	2	2.3	53	21
Normal		0.75	7	4	57.0	38-56	4	60-196	1	14.2	0	0	6	0
Normal		1.0	5	1	20.0	20	0		4	80.0	1*	20.0	0	0
Fasted *ca* 6 hours		0.5	54	33	61.1	20-40	22	60-120	3	5.5	8	14.4	18	25
Histamine	50-180	0	15	0	0	0	0	0	0	0	0	0	15	0
Histamine 15 minutes before adrenalin	12.5	0.5	8	6	75.0	15-23	6	54-75	0	0	0	0	8	0
	25.0	0.5	11	2	18.1	15-30	2	60-150	0	0	0	0	11	0
	50.0	0.5	28	3	10.7	15-50	3	70-130	1	3.6	1	3.6	26	6
	25.0	1.0	16	15	93.7	20-60	3	40-200	0	0	1	6.3	12	3
	35.0	1.0	13	10	76.9	30-90	8	50-230	0	0	1	7.6	9	3
	50.0	1.0	20	8	40.0	15-52	7	98-212	0	0	5	25.0	20	0
Gynergen II, 0.2 mgm.		0.5	8	0	0	0	0	0	0	0	0	0	8	0
Phloridzin, 1 mgm. fasted		0.5	13	0	0	0	0	0	0	0	0	0	13	0

* Died 2 to 3 hours after adrenalin injection.

From the table it may be seen that the injection of adrenalin superimposed upon a mild degree of histamine intoxication modified the toxic effect of adrenalin in three ways:

1. When the proportion of the histamine to that of adrenalin was about 25:1 the incidence of cataract increased 30 to 40 per cent above the maximum caused by the same amount of adrenalin alone. The formation of the opacity was in all respects identical with that described above. There was some indication of increased duration and intensity, but slight differences of this nature in animals with considerable variation are difficult to determine.

2. When the amount of histamine was increased, so that the ratio of the dosages of the drugs definitely exceeded 25:1, the incidence progressively declined.

3. The acute and rapidly fatal systemic reaction usually following such doses of adrenalin did not develop, the mortality dropping and all but one death occurring several hours after the injection. This change resembles alterations in the reaction to adrenalin intoxication observed in rats (15).

Blood sugar. From the glycogenolytic character of the inciting agent and the time of appearance of the opacity, the hyperglycemic reaction seemed to be implicated as the initiating factor of diverse and extensive systemic effects ultimately modifying the stability of lens metabolism. Furthermore in a short series of mice previously treated with ergotamine (Gynergen II, Sandoz) which inhibits adrenalin hyperglycemia, and in fasted mice after a single injection of phloridzin, no opacities could be produced. Therefore an attempt to obtain some comparative figures on the blood sugar in cataractous and non-cataractous mice seemed desirable. Because of the circulatory stasis during adrenalin intoxication reliable peripheral blood samples are not obtainable. The mice were sacrificed for the Hagerdorn-Jensen (HJ) micro-determinations on heart blood specimens. However, some preliminary attempts were made to follow roughly the blood sugar changes using the more rapid Folin-Malmros (FM) micro-method.

In untreated, unanesthetized, fed or semi-fasted mice, tail blood is easily obtained; but because of the excitability and restlessness of the species rapid elevation of the blood glucose occurred frequently, ranging from 68 to 172 mgm. per cent HJ or from 112 to 234 FM. In apparently quiet mice, however, the average for 3 cases was 94 mgm. per cent HJ and for 5 cases 116 mgm. per cent FM.

Blood sugar determinations on heart blood of non-cataractous mice, 20 to 40 minutes after the injection of adrenalin (0.5 mgm.), showed more uniformity between individuals and more agreement between the two methods. In 5 cases the average was 220 mgm. per cent HJ (range—156 to 279), and in 4 cases the average was 225 mgm. per cent FM (range—172 to 292). In cataractous mice under the same conditions the blood sugar in 7 cases averaged 123 mgm. per cent HJ (range—87 to 181), but by the FM method the blood reducing values in 12 cases averaged 412 mgm. per cent (range—252 to 600). According to the Hagerdorn-Jensen determinations, therefore, there appeared to be an unexpected but consistent low "true" glucose level in the cataractous mice during the period when a marked hyperglycemia due to adrenalin should occur, while the high values obtained by the Folin-Malmros method indicated a spectacular rise in the non-glucose reducing fraction.

Discussion. In the adrenalin-induced cataracts in mice the speed of onset, the reversibility, and the opaque, hydrated state of the anterior cortical tissue are grossly similar to the initial stages of cataracts associated with carbohydrate disturbances as described by others (galactose cataracts

in rats—9, 10, 11; naphthalene cataracts—4, 12; certain senile varieties in the diabetic human—9, 13; the rare cataracta diabetica—14). Physiologically they are elicited by the use of a glycogenolytic agent, adrenalin, and facilitated by histamine. A "true" hyperglycemia does not develop, however, as shown by the Hagerdorn-Jensen method. A similar situation has been reported for galactose cataracts (10). The high Folin-Malmros values may indicate a marked increase in the intermediate products of sugar metabolism, since this method is sensitive to non-glucose reducing substances. The significant increase in the incidence of cataract for fasted mice and the fact that histamine may start a demobilization of glycogen (15) and the development of an acidosis also point to acidosis as a possible etiological agent, but no consistent correlation with the incidence of cataract has been proved (cf. 14). The failure to induce opacities in mice after gynergen treatment, which prevents the adrenalin hyperglycemic response and the circulatory collapse, signifies nevertheless the essentiality of carbohydrate participation in the process of opacification.

A second complex of factors, namely, circulatory embarrassment and transfer of fluid to tissues, is according to several authors (9, 14, 16) the fundamental disturbance in diabetic states accounting for the hydrated state of the lens. The sequence of events between the systemic and ocular changes and the dominating extrinsic or intrinsic factors initiating imbibition of water by the lens are not yet clearly established, although the immediate osmotic imbalance and altered permeability may be conditioned by a plethora of carbohydrate metabolites (16), and certain intrinsic physico-chemical changes have been shown to parallel the cataractous process (cf. 5, 12).

Impairment of the circulation and disturbed fluid balance are predominant reactions in adrenalin intoxication (2, 8); and the marked vasodilatation of the uveal tract, strikingly visible in the eyes of albino mice, indicates that the ocular tissues are no exception to the systemic reaction. Furthermore, cataracts appear with the signs of anhydremia, and clear as circulatory improvement and diuresis begin. Hence the effect of histamine in increasing the incidence of opacities may well be due to an intensification of this second factor by its characteristic action on capillary dilatation and permeability, in addition to its possible effect on the carbohydrate cycle. That a combination of acute carbohydrate disturbance with the circulatory changes is essential for opacification is shown by the fact that histamine alone, or in amounts which offset the predominance of the adrenalin effects, leads to no opacification of the lens as the shock syndrome develops. Otherwise, rapid transitory opacities should be induced during the developmental stages of histamine shock, or their incidence should be increased rather than decreased as the ratio of histamine to adrenalin exceeds 25:1 (see table). Therefore it appears that conditions

which set up an acute glycogenolytic reaction (e.g., adrenalin intoxication), but not necessarily simple hyperglycemia, can lead to temporary changes in the stability of the lens and its capsule if there is an impairment of the circulation sufficient to interfere with complete oxidation of the products of carbohydrate breakdown and/or osmotic adjustments. A condition which aggravates either factor (or both, e.g., histamine) can facilitate the lens changes, although it may not be able to initiate them when acting alone.

SUMMARY

1. Reversible subcapsular lens opacities are produced in mice after single intraperitoneal injections of toxic but non-fatal amounts of adrenalin.

2. Moderate doses of histamine administered a few minutes before the injection of adrenalin increase the incidence of opacities and reduce the mortality of lethal doses of adrenalin.

3. The process of opacification appears to be related to some aspect of the carbohydrate disturbance (other than a simple elevation of the circulating glucose) and to the concomitant temporary circulatory impairment.

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A COMPARISON OF THE EFFECTS OF COLD AND THRYROTROPIC HORMONE ON THE THYROID GLAND

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Refrigeration of rats increases the activity of the thyroid gland. This fact has been experimentally shown by several investigators. Seidell and Fenger (1) in 1912 analyzed the iodine content of thyroid glands during summer and winter. They found evidence of iodine storage during summer and depletion in winter. Mills (2) in 1918 observed increased thyroid activity in rabbits, guinea pigs and cats after exposure to low environmental temperatures. Cramer (3) in 1920 reported observations on rats and mice after refrigeration and concluded that the low temperature produced experimental hyperthyroidism. Riddle (4) in 1927 reported that the thyroid gland of the pigeon is more active in winter than in summer. Benedict and McLeod (5) found higher oxygen consumption in animals living in the cold than in animals at room temperature. Mason (6) corroborated Benedict and McLeod's results on human subjects living in different climates. Kenyon (7) reported thyroid hypertrophy as a result of refrigeration of his animals. Kuschinsky (8), later in 1933, and Woitkewitsch (9) in 1935 confirmed Kenyon's work; and Wolf and Greep (10) reported similar results in 1937. Ring (11, 12) showed that the body temperature of animals increased when they were exposed to cold and that to demonstrate hypertrophy of the thyroid, a refrigeration period of three weeks was necessary. Uotilla (13, 14, 15, 16) has applied the cold-stimulus mechanism recently in determining the rôle of the sympathetics, anterior pituitary and hypothalamus in thyroid function, and has measured the increase in height of the thyroid epithelium. Other investigators have used various methods to determine the changes in the thyroid, namely, oxygen consumption, iodine storage, gross estimation of a microscopic picture, and weight gain of the glands.

METHOD. We applied the method of Starr and Rawson (17) to the assay of our specimens. We believe this is a more strictly quantitative procedure than any of the methods cited above. Tissues were removed from the animals and preserved in formalin fixative for 24 hours. They were then prepared for histological examination. The sections were examined under low power; the mean cell height was then measured under oil im-

mersion. The microscope was equipped with an eschelon ocular micrometer (Leitz) calibrated in micra. In measuring the cell height each acinus is observed. The average cell height is judged by eye and measured by the ocular micrometer. This measurement is recorded. Two hundred such acini are measured and the arithmetical mean cell height (M.C.H.) is determined.

Rats were exposed to the temperature of an ice refrigerator which was equipped with a double insulating side panel of glass, allowing a full view of the ice and cold chamber in which the rats were caged. The refrigerator was placed in a bright room. Air circulation was automatic by means of convection currents. The ice was placed in a chamber above the rat cages. A pipe equipped with a loose-fitting connection in which small holes were punched to allow correct exchange of air, drained the ice-chamber. The temperature of the refrigerator ranged from 12° to 17°C. Our refrigerator was never colder than 12°C., whereas the temperature of an electric refrigerator such as was used by former investigators, is maintained near freezing. We believe this temperature difference to be of great importance. The conditions of our experiment give the thyroid a moderate stimulus, producing gradual changes that can be measured. The relatively greater coldness of the electric condensing refrigerator produces violent changes in the thyroid. The animal, especially if handicapped by a complicating operation, can not survive more than a few days. The diet upon which the rats throve was a mixture of milk powder 4 parts, whole wheat flour 4 parts, and an equal amount of calcium carbonate and uniodized sodium chloride $\frac{1}{4}$ part. The time of cold exposure varied from 3 to 56 days. At the end of this period the rats were weighed, killed with ether inhalation and the thyroid glands removed.

Table 1 is a compilation of mean cell heights measured from the thyroids taken from control rats and rats which were refrigerated for varying periods of time, namely, 3, 7, 14, 18, 21, 28, 35, 45 and 56 days. The animals maintained at room temperature had an average cell height of 5.28 micra. Refrigeration for three days produced a limited but definite hypertrophy, the mean cell height for 10 animals averaging 5.90 micra. Refrigeration for 11 more days showed no further effect on the cell height which at that time (14 days) averaged only 5.87 micra, the average being calculated from measurements on eight rats. On the 18th day of refrigeration the cell height averaged from 7 animals was 7.95 micra. This is a marked secondary hypertrophy, which remains more or less stationary until the 45th day when the reaction is at a maximum, with six animals averaging a cell height of 9.08 micra. Between the 45th and 56th days the average cell height dropped from 9.08 micra at 45 days to 6.23 micra at 56 days. Seven rats were included in the 56 day series. This level at 56 days closely approximates the hypertrophy seen after three days of cold exposure.

The colloid gradually disappears until the 18th day when all that remains is a fine reticulum which in most cases continues in this state until after the 45th day when the colloid begins to reappear. After refrigeration for 56 days involution with colloid storage is greater in some animals than in others. The measurements of these changes are given in table 1 and illustrated in figures 1 to 6—photomicrographs of the glands of animals killed after varying periods of time; figure 1 is a section of a room temperature control rat; figure 2 is a section of the gland of a rat refrigerated for 3 days; figures 3 to 6 are pictures of glands autopsied at 14, 21, 45 and 56 days.

To investigate the rôle of iodine in this cycle of events we refrigerated 17 rats for 21 days. To one group of 3 rats, after preliminary ice-box treatment of 24 days' duration, we administered iodine daily in doses of 10,000 gamma, for three days in the form of potassium iodide solution in-

TABLE 1

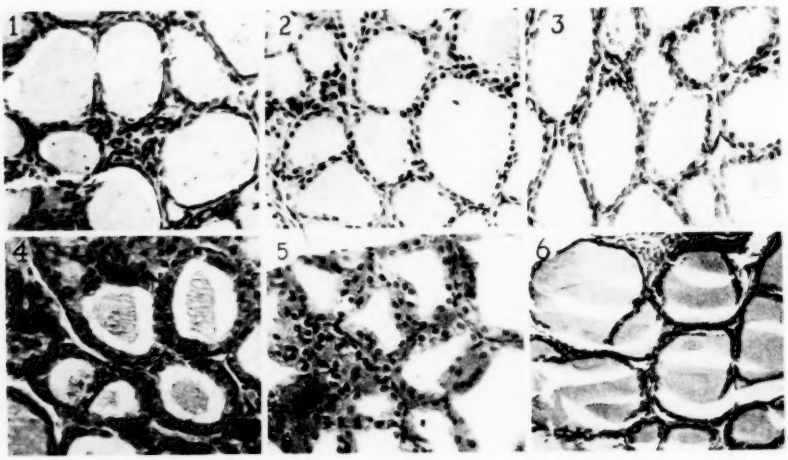
Rat thyroid mean cell height in micra after increasing duration of refrigeration at 15°C.

REFRIGERATION TIME	NUMBER	RANGE	AVERAGE MEAN	STATISTICAL MEAN	CRITICAL RATIO
days					
Room temp.	11	4.97– 5.98	5.28	5.323	
3	10	4.80– 6.60	5.90	5.825	11.1
7	6	5.57– 6.43	6.08	5.908	11.2
14	8	5.42– 6.56	5.87	5.891	11.7
18	7	6.64– 9.47	7.95	8.091	52.1
21	11	6.61– 9.36	7.89	7.964	52.4
28	8	5.26– 9.16	7.45	7.473	33.9
35	5	7.11– 8.35	7.80	7.797	39.2
45	6	8.0 –10.28	9.08	9.016	60.6
56	7	5.34– 7.10	6.23	6.224	18.8

traperitoneally, and then killed the rats. The cell heights averaged 8.81 micra. Another group of 3 rats was refrigerated for 21 days and then injected with potassium iodide in daily doses of 2000 gamma for a period of 12 days. They were autopsied on the 33rd day. The mean cell heights of these animals averaged 5.73 micra. Still another series of 9 rats was refrigerated for 21 days. Injections of sodium iodide solution of different concentrations were begun on the third day of refrigeration; three of the group were injected intraperitoneally with 10 gamma daily doses of sodium iodide; three received 1000 gamma of sodium iodide intraperitoneally per day; and the third group of three rats was given daily injections intraperitoneally of 10,000 gamma. The concentrations were such that each animal received 1 cc. per day. On the 21st day the rats were autopsied, 21 days' refrigeration being sufficient without iodine supplements to cause the secondary hypertrophy. The average thyroid mean cell height of the

animals of this series receiving 10 gamma of potassium iodide was 6.85 micra; that of those receiving 1000 gamma was 5.93 micra; and that of those receiving 10,000 gamma of sodium iodide was 5.18 micra.

A further series of 8 rats was injected with sodium iodide. The entire series was given pre-refrigeration treatment with sodium iodide, three rats receiving 10 gamma per day; two, 1000 gamma daily; and three receiving 10,000 gamma per day. The injections were continued for 26



Figs. 1 to 6. Photomicrographs showing histological changes in the thyroid produced by continuous refrigeration at 12 to 17°C.

Fig. 1. Control.

Fig. 2. Three days' refrigeration, showing some loss of colloid and a small amount of hypertrophy of the acinar cells.

Fig. 3. Fourteen days' refrigeration: The cells remain the same height but the colloid has been lost from the acinar lumen.

Fig. 4. Twenty-one days' refrigeration: Colloid reticulum only remains; cells definitely more hypertrophic.

Fig. 5. Forty-five days' refrigeration: Cells markedly hypertrophic.

Fig. 6. Fifty-six days' refrigeration: Involution of hypertrophy; cells are lower in height; colloid storage has occurred.

days. On the fifth day of treatment the animals were placed in the refrigerator where they remained for 21 days. At the end of this time they were killed and the thyroid studied. The mean cell height of the animals receiving 10 gamma was 6.26 micra; that of those receiving 1000 gamma averaged 6.85 micra; and that of those receiving 10,000 gamma was 6.22 micra (see table 2). All of these animals were refrigerated an adequate length of time to allow a maximum hypertrophy of the acinar cells, that is, a mean cell height of 7.95 micra, as noted after the 18th day of refrigera-

tion. In all but three of these animals the thyroid epithelium was lowered at least to the level noted at three days of refrigeration; the three which remained high were injected only three days with a dosage of 10,000 gamma of potassium iodide, after secondary hypertrophy had been produced with iodine. This time factor probably played a dominant rôle in the submaximal response to the iodine administered. In the group of animals in which the cell height was markedly reduced, three showed reduction to a level the same as that of the room temperature control rats; the remaining 17 rats (73.9 per cent) showed cell counts approximating the level of the primary slight hypertrophy noted after three days' refrigeration.

These experiments suggest that even a large dosage of iodine over a short period of time is insufficient to lower the cell height in a thyroid gland which histologically shows the picture of secondary hypertrophy. They further suggest that the small amount of hypertrophy noted from 3 to 14

TABLE 2

The effect of iodine on rat thyroid mean cell height during refrigeration

GROUP	NUMBER OF ANIMALS	REFRIGERATION TIME	IODINE DOSAGE	NUMBER OF DAYS TREATED	MEAN CELL HEIGHT
		days	*gamma*		*micra*
I	3	27	10,000	3	8.81
	3	33	20,000	12	5.73
II	3	21	10	19	6.85
	3	21	1,000	19	5.93
	3	21	10,000	19	5.18
III	3	21	10	26	6.26
	2	21	1,000	26	5.85
	3	21	10,000	26	6.22

days of refrigeration is not due to iodine deficiency but to a functional activity of the thyroid gland in response to adequate stimulus; but that the further increase in cell height seen after 18 days is due to a deficiency in the supply of iodine to the animal. The increased cell height before depletion of colloid, that is, from 3 to 14 days, is an indication of an increased rate of thyroid hormone formation in response to need created by refrigeration. The additional secondary hypertrophy after colloid depletion (from 18 to 45 days) is not an index of a still further increased rate of thyroid hormone production since it can be prevented by providing iodine. The cold environment at 18 days is the same as that at 14 days. There is, therefore, no greater need for thyroid hormone at 18 or 45 days than there was from 3 to 14 days.

One would expect the same type of early plateau in animals injected with increasing amounts of thyrotropic hormone. In examining Starr and Rawson's curve (17), made from the measurements of guinea pigs given

Antuitrin-T (Parke, Davis),[1] one dose per day for three days at room temperature, the animals being killed on the fourth day, an increase in cell height is observed as the dosage is increased (fig. 7).

An Antuitrin-T dosage of 0.0025 cc. increased the mean cell height 0.69 micra over the control level of 3.93 micra. An increase in dosage of 0.0025 cc. to 0.005 cc. increases the cell height by an additional 0.46 micra. This is double the original dose, or a 100 per cent increment. Now, if the dosage is again doubled, to make the daily injection 0.01 cc. of Antuitrin-T, the actual increase in injected hormone is 0.005 cc. or twice that of the former increment, but the reaction is still only sufficient to increase the mean cell height another 0.52 micra. A daily dosage increase from 0.01 to 0.02 cc. is an increase of 0.01 cc. per injection and is twice the increment of the

TABLE 3

Guinea pig thyroid mean cell height with increasing dosage of thyrotropic extract acting for three days

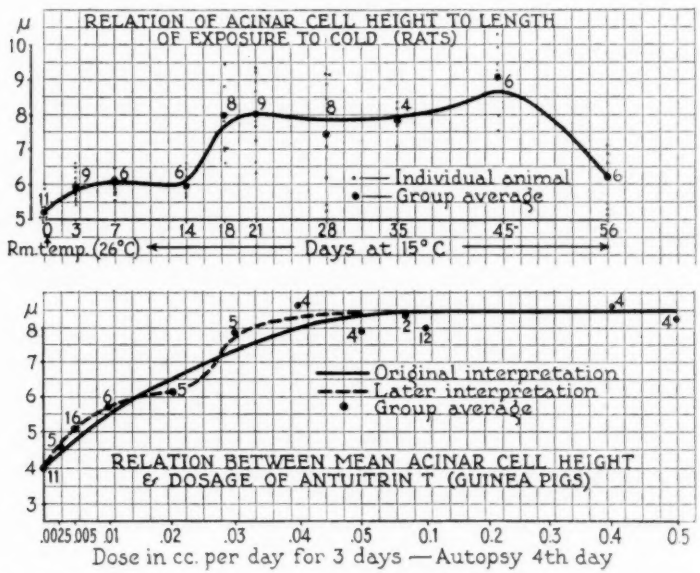
ANTUITRIN-T DOSAGE	DOSAGE INCREMENT	CELL HEIGHT	CELL HEIGHT INCREASE
cc.		*micra*	
Control		3.93	
0.0025	0.0025	4.62	+0.69
0.005	0.0025	5.08	+0.46
0.01	0.005	5.60	+0.52
0.02	0.01	6.09	+0.49
0.03	0.01	7.96	+1.87
0.04	0.01	8.50	+0.54
0.05	0.01	7.87	−0.63
0.085	0.035	8.28	+0.41
0.1	0.015	8.02	−0.26
0.5	0.400	8.23	+0.21

former case. This dosage of 0.02 cc. increases the mean cell height an additional 0.49 micra. Then, if the daily dosage is increased from 0.02 to 0.03 cc., an increment of the same amount as between 0.01 and 0.02 cc., the cell height increase is 1.87 micra or nearly four times the increase seen with the same increments but small total dose as reported above. Further, increase of daily dosage above 0.03 cc. results in no further increase in cell height.

The interpretation of this series of experiments was that as the dosage of Antuitrin-T was increased, the mean cell height increased steadily until a maximum cell height was reached, at which time an excess of Antuitrin-T failed to produce further increase. With our interpretation that the

[1] Generously provided by Dr. E. A. Sharp of Parke, Davis and Company.

greater hypertrophy is an index of iodine depletion, we may analyze the curve of Starr and Rawson and find that if a line is drawn touching the points plotted for the mean cell height levels, the curve resembles that drawn from glands of refrigerated animals (fig. 7). Thus, there is a slow increase in cell height until a dosage of somewhere between 0.02 and 0.03 cc. of Antuitrin-T is reached. Doses larger than 0.02 cc. of Antuitrin-T daily cause a response out of proportion to the increase in dosage. The histological picture is one of gradual colloid depletion until after the dosage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RELATION OF ACINAR CELL HEIGHT TO LENGTH OF EXPOSURE TO COLD (RATS)
Individual animal
Group average
Rm. temp. (26°C)
Days at 15° C
RELATION BETWEEN MEAN ACINAR CELL HEIGHT & DOSAGE OF ANTUITRIN T (GUINEA PIGS)
Original interpretation
Later interpretation
Group average
Dose in cc. per day for 3 days — Autopsy 4th day

Fig. 7. Curves drawn from thyroid mean cell height measurements of rats after refrigeration and guinea pigs after three daily injections of Antuitrin-T. Figures on Antuitrin-T curve indicate number of animals used.

of 0.02 cc. Antuitrin-T is reached. A dosage of 0.03 cc. of Antuitrin-T causes complete absence of colloid with very high acinar cells.

This analysis suggests that the cell heights of 3.93 micra to 6.09 micra are an evidence of functional activity, while the ultimate cell heights, 7.96 to 8.50 micra, are evidence of thyroid response in the presence of colloid depletion. The period of observation in these assays, 96 hours, allows depletion to occur in response to the first and/or second injections. When these are of the critical amount found to be more than 0.02 cc. Antuitrin-T the third injection produces the excessive secondary response.

SUMMARY

Eleven rats were killed at different times during the year and a normal cell height of the thyroid epithelium of 5.28 micra was found. The mean cell heights varied from 4.97 to 5.98 micra.

In a series of 24 rats exposed to cold over periods of time ranging from 3 to 14 days, the average cell height of the thyroid glands was 5.95 micra, which is definite but limited hypertrophy of the acinar cells.

After 18 days in the ice-box a group of 7 rats was killed, and the average cell height of this group measured 7.95 micra.

Thirty-seven rats killed between 18 and 45 days showed an average cell height of 8.03 micra.

Seven rats killed after 56 days in the ice-box showed an average cell height of 6.23 micra, which is approximately the level of the small amount of hypertrophy noted after 3 days in the cold environment.

Iodine in amounts as small as 10 gamma per day, while rats are being refrigerated, is sufficient to limit hypertrophy to an amount equal to that noted after 3 days' refrigeration without iodine, but it does not prevent it. Larger doses, up to 10,000 gamma per day for 26 days, do not materially change this cell height but prevent the secondary hypertrophy resulting from 18 to 45 day terms of exposure to this cold environment.

Analysis of the thyrotropic hormone assay curve of Starr and Rawson indicates primary and secondary levels of epithelial hypertrophy, probably indicative of colloid depletion during the three day period of assay.

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MODIFICATION OF THE ELECTRICAL POTENTIAL OF FROG SKIN BY ACETYLCHOLINE

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Fluctuations in the electrical potential of the skin of the frog and other animals produced by stimulation of the cutaneous nerves have been described by several investigators (for references to the early work of Hermann, Engelmann and others cf. Orbeli, 1910). Schwarz (1915) has related these phenomena to features of the psychogalvanic reflex. Motokawa (1939) has recently reported that the potential of frog skin is modified by acetylcholine. This paper came to my attention while the present experiments were in progress. Motokawa, however, did not use a recording potentiometer.

METHODS. For the study of the effects of nerve stimulation a dorsal skin with cutaneous nerves and spinal cord attached was tied inside out on a vertical glass tube (1.4 cm. in diameter) containing Ringer's solution mounted in a beaker of Ringer's. One Ag-AgCl electrode made contact with the negative morphological outside of the skin through the Ringer's in a side arm of the vertical tube. The other Ag-AgCl electrode made contact with the positive morphological inside of the skin through the outer beaker of Ringer's. The electrodes were connected to a Micromax recording potentiometer or a type K potentiometer. The spinal cord was gently lifted by a thread out of the solution and the nerves running to the morphological inside of the skin (inverted on the tube) were stimulated by Ag-AgCl electrodes with a tetanizing current from a Harvard coil (1 dry cell, coil distance usually zero). For the acetylcholine experiments skins were tied on the blunt end of medicine droppers mounted on no. 4 rubber stoppers. The medicine dropper was filled with Ringer's and clamped vertically so that the skin was immersed in a small beaker of Ringer's solution with or without acetylcholine. These simple skin holders permitted testing a large number of skins in the same solution of acetylcholine. A few skins were treated in the glass skin holder previously described (cf. Barnes, 1939a, fig. 1) through which oxygen was bubbled. In all cases the Ringer's was changed just before treatment with acetylcholine to prevent stimulation by fresh medium. The concentration of acetylcholine (0.66 to 1.0 per cent) is recorded in table 1.

The solution was made up immediately before use. The pH of the solutions was taken with a glass electrode to detect any high alkalinity which is known to hydrolyse acetylcholine even in the absence of esterase (cf. Barbour and Dickerson, 1939). The oxygen consumption of skins treated with acetylcholine was measured in Fenn respirometers.

Results. Single induction shocks applied to the nerves had slight effects on the skin potential but interrupted currents lasting from 1 to 60 seconds produced large changes in the skin potential (see fig. 1A). With

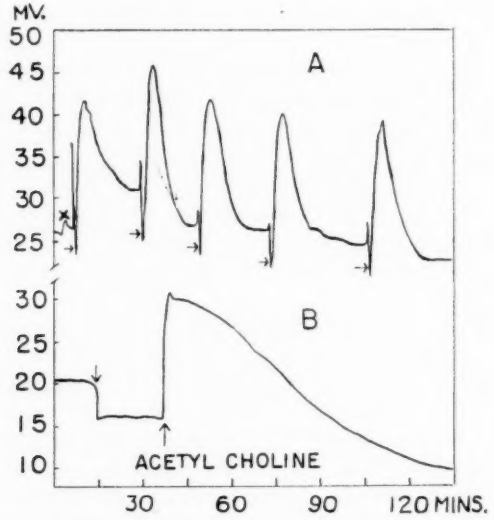

Fig. 1. *A*. The effects of stimulating nerves to the skin on skin potential of the frog. Ordinates: e. m. f. in millivolts (outside surface of skin is negative). Abscissae: time in fifteen-minute intervals. Micromax recorded. The five large peaks follow a 5, 10, 15, 20 and 25 second tetanizing current. The first small wave (marked *x*) following a single break shock. Arrows indicate end of each nerve stimulation. Harvard inductorum: coil distance 0; one dry cell.

B. Effect of 0.70 per cent acetylcholine in Ringer's. At first arrow Ringer's was changed for fresh ordinary Ringer's to eliminate effect of fresh medium.

high potentials (above 50 mv.) the potential usually fell, but with lower potentials the potential frequently increased. The magnitude of the potential change varied from one preparation to another. It is important to note, however, that the results for a given skin were usually constant (see fig. 1A). It was noted that the potential changes always outlasted the stimulus. In many cases the large electrical wave did not begin until the stimulus had ceased (see fig. 1A).

Acetylcholine in a concentration of 0.10 per cent increased the skin

potential when applied to the morphological outside surface (average increase 61.2 per cent, 6 cases) and depressed the potential when applied to the morphological inside surface (average decrease 13.1 per cent, 5 cases). In a concentration of 0.66 per cent acetylcholine on the outside of the skin increased potential 127.7 per cent (6 cases) and on the inside depressed potential 62.0 per cent (5 cases). With 1.0 per cent acetylcholine the average increase after outside treatment was 94.9 per cent (5 cases) and the average decrease after inside application was 60.9 per cent (6 cases). A number of typical experiments are recorded in table 1.

TABLE 1

Effect of acetylcholine on the potential of frog skin

Two preparations from each frog. Numbers 1 and 2 from frog 1; 2 and 3 from frog 2, etc. Frogs weighed 31.0 to 42.5 grams. All males except frogs 1 and 2. o = outside surface treated; i = inside surface treated; a = anterior region of belly; p = posterior region of belly. Temperature taken at every reading, 22–23° C. pH of solutions 7.03–7.70.

NUMBER	DATE	SURFACE TREATED	E. M. F. BEFORE	CONCENTRATION	E. M. F. AFTER	TIME OF ACTION	REMARKS
			mv.	*per cent*	*mv.*	*minutes*	
1	April 6	o. a.	16.3	0.10	19.3	6	18.4% rise
2	April 6	i. p.	37.0	0.10	30.0	5	18.9% fall
3	April 6	o. a.	5.7	0.10	17.0	4	198.2% rise
4	April 6	i. p.	65.6	0.10	58.0	9	11.5% fall
5	April 4	o. a.	2.0	0.66	5.8	1	190% rise
6	April 4	o. p.	8.3	0.66	13.8	5	65.2% rise
7	April 4	i. p.	39.3	0.66	33.7	1	14.2% fall
8	April 4	i. p.	51.0	0.66	29.0	8	43.1% fall
9	April 5	o. a.	11.3	1.0	21.0	4	85.8% rise
10	April 5	i. p.	23.6	1.0	7.0	1	70.3% fall
11	April 5	o. a.	9.0	1.0	25.9	11	187.7% rise
12	April 5	i. p.	35.0	1.0	12.4	8	64.5% fall

Although there is no *a priori* reason that acetylcholine should affect the metabolism of the skin, the O_2 uptake was measured owing to the important though indirect linkage between oxidation and skin potential (for references cf. Barnes, 1939b). The skin from the ventral surface of a frog was divided into three parts and the pieces placed in ordinary Ringer's solution in three Fenn respirometers. The O_2 uptake was measured for a preliminary interval of about one hour. Acetylcholine Ringer's was then introduced into two flasks and fresh Ringer's into the third as a control. The O_2 uptake was again measured for an hour or more. The concentrations of acetylcholine used were 0.066 per cent to 1.0 per cent but no appreciable effect on oxygen consumption was detected. The O_2 uptake of skins in acetylcholine Ringer's fell—18.2 per cent during the

run (8 cases), while the O_2 uptake of control skins in fresh ordinary Ringer's fell—24.4 per cent (4 cases). This represents a possible 6.2 per cent increase in respiration produced by acetylcholine but is probably not significant.

Discussion. The delayed effect on potential of nerve stimulation suggests chemical mediation of the nerve impulse to the skin. The stimulating action of acetylcholine on the outside surface of the skin also supports this hypothesis. The increase in the output of electrical energy produced by acetylcholine is of interest in connection with the problem of potentials arising in muscle and ganglia through the chemical mediation of the nervous impulse. The action of acetylcholine on skin potential, however, probably involves mechanisms essentially distinct from synaptic and neuro-muscular transmission. The persistence of the electrical wave in skin indicates that the choline esterase is not very active in this tissue. It is suggested that the frog skin should prove a particularly useful preparation for the study of the mode of action of acetylcholine on bioelectrical potential. It is possible that acetylcholine mobilizes electrically active ions such as K or Na in the skin, for there is evidence that skin potential is produced by ionic diffusion (cf. Amberson, 1936; Dean, 1939; Barnes, 1940). It is well known that acetylcholine is associated with potassium metabolism (Eccles, 1936). Moreover, Cicardo and Moglia (1940) have shown that acetylcholine liberates potassium from muscle.

The depressing of potential by acetylcholine on the inside surface of the skin is difficult to explain but is not very important for the present discussion, for it is known that the electrical potential is located on the outside surface (cf. Barnes, 1940). Moreover, the skins treated on the inside were reversed on the holder. It was shown (Barnes, 1939a) that contact between the outside surface and the glass holder raises the potential as can be seen in table 1 in which the E. M. F. of skins from the same frog are recorded in normal and reversed orientation. Skins marked "i" in table 1 were treated on the inside and consequently were tied inside out on the holder. These unusually high potentials are probably unstable. The depression may be the result of the high concentration of acetylcholine used in the experiments. Thus 0.66 per cent produced a depression of 77.7 per cent, while 0.10 per cent depressed only 13.1 per cent. The inhibiting action of acetylcholine is well known to depend on concentration in other tissues.

The respirometer experiments indicate that acetylcholine is not altering potential through oxidative mechanisms of the skin. Fifteen minutes elapsed between the introduction of acetylcholine into the flask and the next reading of O_2 uptake. To detect any initial effect which might have been missed during this interval, five experiments were run with flasks having sidearms for tipping in the reagent. The average O_2 uptake

showed an increase of 1.5 per cent over the entire runs and an average of 14.9 per cent increase for the interval 15 to 19 minutes immediately after tipping. (The stop cocks were closed for about 3 minutes during the operation of tipping the respirometer.) The slight increase in O_2 uptake on tipping is probably the result of adding fresh Ringer's solution. The concentration of acetylcholine in these five runs was 0.20 to 0.32 per cent.

SUMMARY

1. Stimulation of the nerves of frog skin preparations with a tetanizing current produces large changes in potential of the skin which always outlast the stimulus.

2. Acetylcholine produces a rise in the electrical potential of frog skin when applied to the outside surface.

3. Acetylcholine has little or no effect on the oxygen consumption of the skin.

This investigation was made possible by a grant from the George Sheffield Scientific Fund.

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ON THE MECHANISM OF THE DEPRESSION OF THE SERUM POTASSIUM LEVEL BY EPINEPHRINE

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It has been shown by Bachromejeu (1), D'Silva (2) and others that intravenous administration of epinephrine results in a brief preliminary increase in serum potassium which is followed by a prolonged fall to below control levels. Insulin also causes a marked lowering of the serum potassium level (Briggs et al. (3), Kerr (4)); an effect which is independent of the presence of the adrenal medulla (5). The possibility that the ultimate decrease in serum potassium following epinephrine administration is at least in part a result of mobilization of insulin has been investigated by D'Silva (2). D'Silva found that pancreatectomy reduced the lowering effect of epinephrine on serum potassium but did not abolish it. In my experience in this connection, the result of pancreatectomy on the subsequent course of epinephrine is unpredictable. Apparently, mechanisms in addition to insulin mobilization are involved.

Fenn and Cobb (6) have shown that a decrease in the plasma potassium concentration can be accomplished by increasing the hydrogen ion concentration within the cell (frog muscle). Of interest in this connection is the observation by Pulver (7) that if glucose be added to a suspension of yeast cells, the potassium concentration of the solution surrounding the cells is markedly lowered as fermentation proceeds. After fermentation is essentially complete the potassium concentration slowly returns to the control level. Simultaneously with the onset of fermentation, a marked, irreversible drop in pH occurs. It is conceivable that potassium enters the cell in response to the pH gradient set up by fermentation and slowly returns with establishment of equilibrium conditions when fermentation is over.

Although it is well known that epinephrine increases the lactic acid content of the blood (8, 9) and lowers the alkali reserve (10), data as to its effect on blood pH are rather incomplete. The effect of epinephrine on blood pH has therefore been investigated in the hope that it might cast some light on the serum potassium changes.

EXPERIMENTAL. All serum potassium and pH measurements were

made on arterial blood. Whenever anesthetics were administered, a period of at least one hour was allowed to elapse for the stabilization of serum potassium and blood pH prior to the epinephrine infusion. Control pH measurements were made at 10 to 15 minute intervals prior to the epinephrine or insulin administration until readings that were constant or varied by no more than 0.01 pH were obtained.

The effect of subcutaneously administered epinephrine (0.5 mgm. per kgm. of body weight) was investigated on unanesthetized dogs. The effect of intravenously infused epinephrine (0.005 mgm. per kgm. of body weight per minute) was investigated on *a*, cats anesthetized with Dial;[1] *b*, eviscerated, hepatectomized cats under Dial anesthesia, and *c*, cats anesthetized with Dial and receiving constant artificial ventilation. For the cats in part *c* blood pH was determined prior to the application of artificial ventilation and subsequently the artificial ventilation was adjusted to give control blood pH readings just slightly above (within 0.03 pH) that maintained by the cat's own respiration. The effect of subcutaneously administered insulin (0.5 unit per kgm. of body weight) was investigated on unanesthetized dogs.

TABLE 1

Effect of epinephrine (0.5 mgm. per kgm. body weight, subcutaneously) on blood pH and serum potassium of unanesthetized dogs

	BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS AFTER EPINEPHRINE EXPRESSED AS AVERAGES OF RESULTS ON 3 DOGS			
	15 minutes	30 minutes	60 minutes	120 minutes
pH	−0.01	−0.02	−0.07	−0.12
Serum K in millimols per liter	−0.43	−0.69	−1.12	−1.28

All pH measurements were made with the glass electrode. Potassium determinations were made on serum by the method of Kramer and Tisdall (11).

RESULTS AND DISCUSSION. The effect of subcutaneously administered epinephrine on blood pH and serum potassium of unanesthetized dogs is shown in table 1. A simultaneous lowering of both serum potassium and blood pH occurred. However, no strict proportionality between the two is evident.

Because of possible variations in the rate of absorption of subcutaneously injected epinephrine and the desirability of having more exact information on the immediate effects of epinephrine, a series of experiments was performed on anesthetized cats into which epinephrine was infused intravenously at a constant rate for 30 minutes. The results of these experi-

[1] Supplied through the courtesy of Ciba Pharmaceutical Products, Inc.

ments are shown in table 2. Two types of pH changes were observed; and, for this reason, the animals have been divided into two groups. In 5 of the 8 cats used, blood pH fell promptly reaching a new level in approximately 20 minutes, followed by a return to near control levels within 30 minutes after cessation of the infusion. The other 3 cats showed a preliminary rise in pH followed by the fall to below control levels.

The serum potassium changes showed no significant variations between the two groups and have therefore been included as representative of the 8 cats. The initial increase due to release of potassium from the liver is present. Whether the initial rise in pH occasionally observed bears any relation to the mechanism by which epinephrine effects a release of potassium from the liver is not known and the present technic is not suitable for further inquiry along this line. Following the initial rise in serum potassium the fall to below control levels occurred. An additional, and

TABLE 2

Effect of epinephrine (0.005 mgm. per kgm. per minute, intravenously) on blood pH and serum potassium of anesthetized cats

	BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS DURING EPINEPHRINE INFUSION EXPRESSED AS AVERAGES					BLOOD pH AND SERUM K CHANGE AT INTERVALS FOLLOWING CESSATION OF EPINEPHRINE INFUSION EXPRESSED AS AVERAGES		
	2 minutes	7 minutes	12 minutes	20 minutes	30 minutes	10 minutes	20 minutes	30 minutes
pH, 5 animals	−0.03	−0.09	−0.12	−0.13	−0.13	−0.07	−0.04	−0.02
pH, 3 animals	+0.04	+0.01	−0.06	−0.08	−0.09	−0.06	−0.03	−0.01
Serum K in millimols per liter	+4.60	+1.53	+0.01	−1.02	−1.03	−3.07	−1.79	−1.41

to the author's knowledge a hitherto unrecorded, feature occurred on cessation of the epinephrine infusion. Instead of the serum potassium immediately beginning to return to control levels, there resulted a sharp secondary decrease followed by the process of gradual recovery.

As stated above, an initial effect of epinephrine is to bring about a release of potassium from the liver. Apparently, at the end of the epinephrine infusion the liver rapidly recovers its potassium stores at the expense of the extracellular fluids. To test this, the effect of epinephrine on the serum potassium of eviscerated, hepatectomized cats was investigated. Results of experiments on 3 cats are presented in table 3.

In the absence of the liver, the initial rise in serum potassium does not occur. The fall in serum potassium begins immediately; and, on cessation of epinephrine administration, the return of serum potassium toward control levels is not interrupted by a secondary fall. The rapidity with which the liver, deprived of some of its potassium, picks up potassium from

the extracellular fluids is an additional factor in the ultimate fall in serum potassium under the influence of epinephrine. Incidentally, this also may explain why Heppel (12) found no significant decrease in liver potassium in his potassium deprived rats.

It will be noted in table 3 that during epinephrine infusion blood pH fell promptly to a lower level but differed from the experiments on the intact cat in that on cessation of the infusion recovery was much more prompt. For some unknown reason these animals showed a marked

TABLE 3

Effect of epinephrine (0.005 mgm. per kgm. body weight per minute intravenously) on blood pH and serum potassium of anesthetized, eviscerated, hepatectomized cats

	BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS DURING EPINEPHRINE INFUSION EXPRESSED AS AVERAGES				BLOOD pH AND SERUM K CHANGE AT INTERVALS FOLLOWING CESSATION OF EPINEPHRINE INFUSION EXPRESSED AS AVERAGES		
	2 minutes	7 minutes	12 minutes	20 minutes	10 minutes	20 minutes	30 minutes
pH	−0.04	−0.07	−0.09	−0.09	+0.04		
Serum K in millimols per liter	−0.13	−0.26	−1.12	−1.28	−0.85	−0.54	−0.33

TABLE 4

Effect of epinephrine (0.005 mgm. per kgm. per minute, intravenously) on blood pH of anesthetized cats receiving constant artificial ventilation

	BLOOD pH CHANGE AT VARYING INTERVALS DURING EPINEPHRINE INFUSION EXPRESSED AS AVERAGES					BLOOD pH CHANGE AT VARYING INTERVALS FOLLOWING CESSATION OF EPINEPHRINE INFUSION EXPRESSED AS AVERAGES		
	2 minutes	7 minutes	12 minutes	20 minutes	30 minutes	10 minutes	20 minutes	30 minutes
pH, 3 animals	−0.03	−0.11	−0.14	−0.16	−0.17	−0.10	−0.06	−0.03
pH, 1 animal	+0.02	−0.03	−0.05	−0.05		0.00		

acceleration in respiratory rate on cessation of the epinephrine infusion, whereas the intact cats did not.

To determine if variations in respiration might have a rôle in the observed blood pH changes following epinephrine, 4 cats were given constant artificial ventilation throughout the experiment. The results are recorded in table 4. In 3 of these animals blood pH fell promptly and markedly during the epinephrine infusion, while in one a slight rise in pH was followed by only a slight fall. The degree and character of the pH changes in these experiments compare favorably with those observed in intact, anesthetized cats (table 2). Apparently change in pulmonary ventilation is

not a causative factor in the blood pH changes during epinephrine infusion into intact, anesthetized cats and the pH changes probably have their origin within the cell. Recent experiments by Griffith, Emery and Lockwood (13) lend further support to this assumption. These workers found that epinephrine, in doses equivalent to those employed here, causes a small but definite increase in pulmonary ventilation. This effect would be opposed to the blood pH decrease observed here.

Artificial ventilation experiments with eviscerated, hepatectomized cats were not successful. Apparently this was due to lack of reserve blood supplies in these animals. While it is possible to equilibrate fairly well their blood pH under artificial ventilation, withdrawal of quantities of blood for analysis in the absence of reserve supplies in itself alters the pH balance in a manner that cannot be compensated for in this type of experiment.

Although insulin has been previously reported as having no demonstrable effect on blood pH, in view of its pronounced effect on serum

TABLE 5

Effect of insulin (0.5 unit per kgm., sucutaneously) on blood pH and serum potassium of unanesthetized dogs

	BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS AFTER INSULIN EXPRESSED AS AVERAGES OF RESULTS ON 3 DOGS		
	30 minutes	60 minutes	120 minutes
pH	+0.02	+0.02	0.00
Serum K in millimols per liter	−0.38	−0.67	−1.28

potassium, it was felt that this experiment might bear repetition. Results on 3 dogs are recorded in table 5. Serum potassium decreased markedly but no significant changes occurred in blood pH. The mechanism by which insulin exerts its effect on serum potassium is not well known. However, the simultaneous decrease that occurs in serum phosphate may have some bearing in this connection.

SUMMARY

The action of epinephrine in effecting a decrease in serum potassium cannot be completely explained as being a result of secondary mobilization of insulin. It has been shown here that epinephrine causes a marked lowering in blood pH, an effect which very probably has its origin in an increase in the hydrogen ion concentration within the cell. By analogy to Fenn and Cobb's findings (6) that increases in the hydrogen ion concentration within the cell lead to a lowering of the potassium concentration in the extracellular fluids, it is suggested that this may explain in part the decreases in serum potassium observed following epinephrine.

An additional factor in the ultimate fall in serum potassium is the rapidity with which the liver replenishes its stores of potassium at the expense of the extracellular fluids following cessation of epinephrine administration.

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THE EFFECT OF VARIOUS AGENTS ON THE BLOOD PRESSURE OF RENAL HYPERTENSIVE DOGS[1]

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Bilateral constriction of the renal arteries leads to a persistent hypertension in dogs (1) which in many respects resembles essential hypertension in man. The similarities include a normal cardiac output, blood volume, blood viscosity, and hematologic picture; normal blood chemistry and renal function tests except in the malignant phase; a failure to respond significantly to various operative procedures on the sympathetic nervous system; a reduced renal blood flow; an increased peripheral resistance; arteriolosclerosis in various organs; and an increased renin content of the kidneys (2, 3). We, therefore, studied a group of active principles and organ and plant extracts which have been reported to be of value in the treatment of essential hypertension to determine their effect on the blood pressures of renal hypertensive dogs. These included estrone, testosterone, and extracts of liver, pancreas, and garlic and parsley. Obviously, if the results with these substances resembled those obtained in essential hypertension, the findings would constitute further evidence for a parallelism between experimental renal hypertension and essential hypertension. On the other hand, a difference would be evidence against a similarity of the two conditions.

The pathogenesis of experimental renal ischemic hypertension still remains unsolved. Inconclusive evidence points to the release of a humoral agent from the ischemic kidney (2) but whether it acts directly or otherwise on the vessels to increase the peripheral resistance is not known. Consequently we studied the effect on the blood pressures of renal hypertensive dogs of a group of extracts prepared from organs definitely or possibly involved in the pathogenesis of experimental renal hypertension. The preparations included in this second group were renin, whole kidney, adrenal cortical extract, whole pituitary extract, and pituitrin.

During the course of the work an opportunity was afforded for observing the effect of distemper and of extensive cellulitis on the blood pressures of renal hypertensive dogs.

[1] This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

METHOD. Dogs weighing 9 to 12 kgm. were subjected to bilateral constriction of the renal arteries by the Goldblatt technique (1, 2) and the resulting hypertension was permitted to stabilize over a period of three or four months. In some cases further constriction of the arteries proved necessary in order to sustain the hypertension (2). Mean blood pressure readings by the method of Dameshek and Loman (4) were obtained from the femoral artery three times per week. Blood urea determinations and urinalyses were made when indicated. The study of each preparation involved a preliminary control period of one or two months during which the blood pressure of the animal was determined to be relatively constant. This was followed by an experimental period during which the preparation was administered daily in most cases for one month. Subsequently there was another control period of one or two months. Some of the animals were later used for the study of other active principles or extracts. Parallel observations were made on normal dogs with several of the substances.

RESULTS. *Group 1.* a. Estrone. Daily intramuscular injections of 0.1 mgm. (1000 i.u.) per kilo of estrone[2] into one hypertensive dog for one month had no significant effect on the blood pressure of the animal.

b. Testosterone. Daily intramuscular injections of 2.5 mgm. per kilo of testosterone[3] into one hypertensive dog for one month did not appreciably influence the pressure levels.

c. Liver extract. A peptone-containing aqueous extract of liver[4] said to contain none of the known depressor substances was tested in two hypertensive dogs. One of the dogs was given 2 cc. of the extract intravenously twice daily and intramuscularly once per day for one month. The other animal was subjected to the same regimen with twice the foregoing dosage. There was no significant change in the blood pressures of either dog as a result of the liver extract injections.

d. Pancreatic extract. A preparation containing desiccated pancreas and thyroid[5] in a ratio of 25:1 was administered orally to one hypertensive dog in a dosage of 0.3 gram daily for one month. The pressure levels of the animal were unaltered.

e. Garlic and parsley. A preparation of desiccated garlic and parsley was administered orally to one hypertensive dog in a dosage of approximately 2 grams daily for two months without any significant change in the blood pressure.

[2] Theelin, supplied by Parke, Davis and Company, Detroit, Michigan (Dr. Oliver Kamm).

[3] Perandren, supplied by Ciba Pharmaceutical Products, Summit, N. J. (Dr. R. MacBrayer).

[4] Supplied by The Armour Laboratories, Chicago, Illinois (Dr. E. F. Pike).

[5] Panlittol, supplied by The Armour Laboratories, Chicago, Illinois (Dr. E. F. Pike).

The results with this first group of hormones and extracts are summarized in table 1.

Group 2. a. Renin. One hypertensive and one normal dog were given daily intramuscular injections of dog renin (equivalent in acute pressor effect intravenously to approximately 4 units of pituitrin) for two and one-half months. A similar pair of dogs received daily intravenous injections of dog renin for one month in twice the foregoing dosage. The two hypertensive dogs showed average increases of 10 and 15 mm. of Hg

TABLE 1

The effect of various agents on the blood pressure of renal hypersensitive and normal dogs

EXPERIMENT NUMBER	ACTIVE PRINCIPLE OR EXTRACT	DAILY DOSAGE	ROUTE OF ADMINISTRATION	PERIOD OF TREATMENT	BLOOD PRESSURES*			
					Before constriction of renal arteries	Before treatment	During treatment	After treatment
1	Estrone	0.1 mgm./kgm.	i.m.	1 mo.	138-172/152	180-202/186	168-198/181	160-204/184
2	Testosterone	2.5 mgm./kgm.	i.m.	1 mo.	138-172/152	170-190/182	170-192/187	168-208/188
3	Liver extract	6 cc.	i.v., i.m.	1 mo.	140-152/146	149-170/166	157-190/172	152-192/164
4	Liver extract	12 cc.	i.v., i.m.	1 mo.	132-136/134	158-180/170	162-188/172	170-188/178
5	Pancreatic extract	0.3 gram	Per os	1 mo.	140-165/148	168-190/174	168-194/182	170-190/180
6	Garlic and parsley	2.0 grams	Per os	2 mo.	140-165/148	155-182/170	160-177/172	160-186/168
7	Renin	0.5 gram/kgm.	s.c.	2½ mo.	121-160/142	168-216/190	186-218/208	190-207/197
8	Renin	1.0 gram/kgm.	i.v.	1 mo.	111-140/127	160-184/172	180-195/188	153-186/173
9	Whole kidney	250 grams	Per os	1 mo.	138-172/152	172-204/192	172-212/196	180-206/198
10	Whole kidney	250 grams	Per os	1 mo.	112-118/115	160-182/172	164-182/176	162-194/180
11	Adrenal cortical extract	0.1 cc./kgm.	s.c.	1 mo.	111-140/127	154-175/168	156-182/166	160-184/172
12	Whole pituitary extract	1.5 grams/kgm.	i.m.	1 mo.	121-160/142	192-210/199	192-202/200	170-208/195
13	Pituitrin	1 cc./kgm.	i.m.	2 wk.	121-160/142	170-215/195	182-196/190	190-200/195
14	Pituitrin	1 cc./kgm.	i.m.	2 wk.	140-165/148	160-186/168	165-180/172	164-190/176
15†	Renin	0.5 gram/kgm.	s.c.	2½ mo.		112-130/122	122-150/134	122-127/125
16†	Renin	1.0 gram/kgm.	i.v.	1 mo.		134-148/143	145-172/154	140-150/145
17†	Adrenal cortical extract	0.1 cc./kgm.	s.c.	1 mo.		126-148/140	132-148/141	134-148/143

* Numerator figures denote extremes; denominator denotes average for period.

† Nonhypertensive dogs.

respectively in their blood pressures during the periods of injection. However, following the cessation of the injections the blood pressures fell to their preinjection hypertensive levels. Each of the normal dogs likewise showed a temporary average increase of approximately 10 mm. of Hg during the renin injection period.

b. Whole kidney. Two hypertensive dogs were given 250 grams of fresh hog kidney daily by mouth for one month without any significant effect on blood pressure.

c. Adrenal cortical extract. An active adrenal cortical extract[6] was administered subcutaneously daily for one month to one hypertensive and one normal dog in a dosage of 0.1 cc. (7.5 Swingle dog units) per kilo. There was no change in the blood pressures.

d. Whole pituitary extract. An aqueous extract of whole pituitary[7] was given daily intramuscularly for one month in a dosage of 1.5 grams of fresh gland equivalent per kilo to one hypertensive dog without any significant change in blood pressure.

e. Pituitrin. A standard solution of posterior pituitary[7] in a daily dosage of 10 U.S.P. units per kilo intramuscularly to three hypertensive dogs for two weeks did not significantly alter the blood pressures of the animals.

The results for this second group of extracts are summarized in table 1.

Infections. As previously reported (5) the appearance of distemper in four hypertensive dogs produced a prompt drop in the blood pressures to normal or subnormal levels. When recovery occurred, the hypertension returned. On the other hand an extensive mixed streptococcus and staphylococcus cellulitis in four hypertensive dogs did not lower their blood pressures.

Discussion. Estrogens and testosterone have both been reported to be of value in reducing the blood pressure in essential hypertension (6, 7). However, the best clinical evidence indicates that these hormones have no significant effect on the blood pressure in essential hypertension (8, 9) except in patients with complicating climacteric symptoms (10). From the above results, it is apparent that estrone and testosterone in dosages 7 to 10 times those employed in the treatment of essential hypertension had no significant effect on the blood pressure of experimental renal hypertension in dogs. The results with these two hormones in this form of experimental hypertension therefore agree substantially with those obtained by carefully controlled observations in essential hypertension.

As already stated, therapeutic claims for an antihypertensive effect in essential hypertension have been made for extracts of liver (11), pancreas (12), and garlic and parsley (13). However, properly controlled evidence has shown that these and similar agents have no significant effect on the blood pressure of this condition other than that obtained with a properly administered placebo (3, 9). There was likewise no significant effect from these extracts on the blood pressures of our renal hypertensive dogs even though on a weight basis the dosages administered were 7 to 10 times those recommended for the human. The negative results with these extracts and the two sex hormones therefore offer a further paral-

[6] Supplied by the Wilson Laboratories, Inc., Chicago, Illinois (Dr. David Klein).
[7] Supplied by The Armour Laboratories, Chicago, Illinois (Dr. E. F. Pike).

lelism between experimental renal ischemic hypertension and essential hypertension.

There is suggestive indirect evidence that renin is the effective pressor agent released from the ischemic kidney in experimental renal hypertension and essential hypertension (2). The slight but significant temporary increase in blood pressure produced by repeated injections of dog renin as reported above constitutes further indirect evidence for a pathogenetic rôle for renin in experimental renal hypertension.

The known neutralizing effect of normal kidney on the pressor action of ischemic kidney in the intact animal (2) prompted our observation on the oral administration of fresh hog kidney. The negative results obtained may be due to inadequate dosage, ineffectiveness by mouth, or other factors. Very recently the successful extraction of an antihypertensive substance from normal kidney has been reported (14, 15).

The rôle of the adrenal cortex in experimental renal hypertension is still unsettled. Certain investigators have reported that the hypertension is maintained in completely adrenalectomized animals only if minimal amounts of the cortical hormone are supplied (2). Another group, however, found that hypertension can be produced and maintained in adrenalectomized dogs without supportive treatment during the survival period (16). The failure in our hands of a potent adrenal cortical extract to influence the blood pressure in experimental renal hypertension speaks against any direct effect of the adrenal cortex in the pathogenesis of the condition.

The results reported for hypophysectomy in experimental renal hypertension (17) indicate that the pituitary is not directly involved in the pathogenesis of the condition. Our failure to influence the blood pressure of renal hypertensive dogs with large doses of whole pituitary extract and of pituitrin is in keeping with this conclusion and contrasts with the pressor effect observed with dog renin.

The above findings, therefore, point to a possible rôle for renin in the pathogenesis of experimental renal hypertension in the dog and argue against such a rôle for the adrenal cortex and the pituitary. For essential hypertension the available evidence leads to similar conclusions (3).

CONCLUSIONS

1. Estrone, testosterone, and extracts of liver, pancreas, and garlic and parsley had no significant effect on the blood pressures of renal ischemic hypertensive dogs.

2. Dog renin temporarily increased the blood pressures of renal hypertensive and normal dogs slightly but significantly.

3. Fresh hog kidney, adrenal cortical extract, whole pituitary extract, and pituitrin were without effect on the blood pressure in experimental renal hypertension in dogs.

4. Distemper lowered the blood pressures of renal hypertensive dogs but extensive cellulitis was without effect.

5. The growing evidence of similarity between experimental renal ischemic hypertension in the dog and essential hypertension in man is further substantiated.

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THE MECHANISM OF THE ACTION OF DICARBOXYLIC ACIDS IN BLOOD COAGULATION

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The use of sodium citrate as an anticoagulant in blood transfusion led immediately to the study of its effect on the recipient. Weil (1) in 1915 records a 50 per cent reduction in the coagulation time in hemorrhagic conditions following administration of 5 grams of sodium citrate. Neuhof and Hirshfeld (2) attributed the effect of sodium citrate to platelet destruction. In 1934–35, De Souza and Hocking (3) studied the action of sodium citrate in producing a hypercoagulability of blood. They state that the intramuscular injection of sodium citrate rapidly makes the blood more coagulable, and increases the alkali reserve.

Noda (4) reported the action of gallic acid in decreasing coagulation time. A further report in 1939 by Noda (5) extends his original work to include dicarboxylic acids, aromatic acids of various types, and simple fatty acids. He states that the carboxyl is the essential group, and that its effect is increased by the presence of a hydroxyl group.

Steinberg and Brown (6) have further extended this work. They report the efficacy of malonic, maleic, fumaric, d,l-tartaric, glycollic, phthalic, mandelic, citric, sebacic, glutaric, adipic, pyruvic, azelaic, succinic acids, and ethyl and methyl oxalate.

Milliken (7) reports on the use of coagulant preparation containing dicarboxylic acid in transurethral prostatic resection. Davies (8) used intravenously administered oxalic acid in the control of hemorrhage in veterinary surgery.

Sterner and Medes (9) found that the *in vitro* addition of cysteine, taurine and taurocholic acid to whole blood delayed coagulation. The action of cysteine in inhibiting coagulation is on prothrombin, preventing its activation to thrombin. Putnam and Hoefer (10) also state that cysteine acts as anti-prothrombin.

Dicarboxylic acids in vitamin K deficiency. One of the first questions brought to mind by the foregoing observations is the possible effect of dicarboxylic acids in controlling the hemorrhages resulting from vitamin K deficiency. In investigating this possibility, we have employed chicks and used the technique of Ansbacher (11), using the ether-extracted fish

meal diet. The changes in clotting times were determined by puncture of a wing vein with a fine needle; the blood was allowed to flow into a micro-test tube and its clotting time determined. The acids in physiological saline were given intravenously, intraperitoneally, hypodermically and intramuscularly. The clotting times were re-determined in six hours. Table 1 summarizes the results.

TABLE 1

Anti-hemorrhagic effect of malonic acid in vitamin K deficiency

(Clotting times listed as average value for the group)

CHICKS	DOSE	ADMINISTRATION ROUTE	CLOTTING TIME BEFORE	CLOTTING TIME 6 HOURS
	mgm.			
6	0.25	Intravenous	>8 hrs.	18 min.
6	0.50	Intravenous	>8 hrs.	20 min.
12	0.25	Intramuscular	>2 hrs.	20 min.
6	0.50	Intramuscular	>2 hrs.	11 min.
3	0.12	Intraperitoneal	>1 hr.	14 min.
6	0.25	Intraperitoneal	>2 hrs.	12 min.
6	0.50	Intraperitoneal	>2 hrs.	11 min.
3	0.25	Hypodermic	>1 hr.	25 min.
6	0.5 cc. saline	Intravenous	>2 hrs.	>2 hrs.
3	0.5 cc. saline at pH 4	Intravenous	22 min.	>1 hr.
3	0.5 cc. saline at pH 4	Intramuscular	>2 hrs.	>2 hrs.
3	0.5 cc. saline at pH 4	Intraperitoneal	>2 hrs.	>2 hrs.

It would seem that the coagulation time of vitamin K deficient birds is reduced to nearly normal values by dicarboxylic acid therapy. As the maximum effect of dicarboxylic acids in the normal animal occurs from $\frac{1}{2}$ to 1 hour after the injection, a redetermination of clotting times was made at varying intervals in an attempt to ascertain the time of maximum effect. Our results in a series of 24 chicks over time intervals from 30 minutes to 7 hours showed no consistency. The maximum effect might be reached in 30 minutes or in 7 hours.

Prothrombin times were determined by the method of Almquist and Klose (12). As it is necessary to sacrifice the chick in order to determine these values, we used two series. One series of untreated vitamin K deficient chicks showed prothrombin times which average 31 minutes for the group of 14. Prothrombin times were taken on the vitamin K deficient treated chicks six hours after the administration of 1 mgm. of malonic acid. The average value for the prothrombin times on this series of 9 chicks was 9 minutes. Normal chick prothrombin time was determined to be 3 minutes. These values demonstrate a pseudo increase in prothrombin levels.

In a smaller series of chicks, oxalic acid was tested for anti-hemorrhagic action in vitamin K deficiency with essentially the same results.

Studies of effect of dicarboxylic acids on coagulation of blood in normal animals. In the course of our investigations on the effect of various substances on coagulation time, we have used rabbits as experimental animals. A sample of blood was taken by cardiac puncture and immediately thereafter the substance to be tested was injected into an ear

TABLE 2

Effect of dicarboxylic acids on coagulation time of blood in normal animals

ACID	DOSAGE	COAGULATION TIME REDUCED TO	COAGULATION TIME PROLONGED
	mgm. per kgm.	*per cent*	*per cent*
Oxalic	0.5–30	10–50	
Oxalic	100		100–300
Malonic	3	20–60	
Succinic	3	25–60	
Glutaric	3	30–70	
Fumaric	3	No effect	
Maleic	3	No effect	
Ascorbic	>10	75–85	
Gluconic	10	No effect	
Mucic	10	No effect	
Sodium acetate	<10	No effect	
Sodium propionate	<10	No effect	
Aspartic	0.5		100–300
Glutamic	0.5		25–50
Cysteine	1.0		90–150
Gallic	<5	No effect	
Salicylic	<5	No effect	
Benzoic	<5	No effect	
Phthalic	5	80–90	
Malic	>100	50–90	
Citric	>100	50–90	
Tartaric	>100	50–90	
Sulfuric	0.5 cc. of 0.03 N		50–200
Hydrochloric	0.5 cc. of 0.03 N		50–200

vein. After a half-hour interval, a second cardiac puncture was made and the coagulation time redetermined. Coagulation times were determined by collecting blood in a small test tube and determining the period elapsing from the moment the blood is shed to the time of congealing, as indicated by the inversion of the tube.

In our study of the mechanism of the action of dicarboxylic acids, it was necessary to observe the effect of a variety of acids on clotting time. The results of these observations are summarized in table 2.

The members of the oxalic acid series have a definite and powerful action in decreasing coagulation times. The activity decreases as the number of carbon atoms increases. This observation is entirely in conformity with that of Noda (5). Malonic acid, because of its greater solubility and lesser toxicity, seems the member of this group to be preferred in therapeutics. Oxalic, malonic, succinic and glutaric acids were tested as representative of this group. Rabbits of 5 lb. weight are used, and in these animals dosages of 1 mgm. of oxalic or malonic acid are effective in reducing the coagulation times to 10 per cent of their original values. Values of from 30 to 300 mgm. per kilogram are given as the fatal dose of oxalic acid, and as the therapeutic dose in reducing coagulation times is less than 1 mgm. per kilogram, it seems to be a safe compound to use. Toxic doses of oxalic acid (100 mgm. per kilo) produce the manifestation of a low calcium tetany and a marked prolongation in clotting time. We noted increases of 100 to 300 per cent in clotting times on blood taken when the animals were in a tetanic seizure. The exact concentration producing a transition from decreased to increased coagulation times is between 40 and 60 mgm.

Results with sodium fluoride. If the oxalate mechanism is primarily one involving calcium and its relationship to clotting, it would seem that the alkali salts of fluorine should have a similar action because the formation of the insoluble calcium fluoride should interfere with the clotting of blood in the same manner as does the formation of the insoluble calcium oxalate. Using the standard 5 lb. rabbits, we gave 0.1 mgm. to 5 mgm. intravenously with no effect on coagulation times. Doses of 10 to 60 mgm. decreased the coagulation time to as low as 30 per cent of the original value. Dosages of 200 mgm. and higher prolong the clotting times. A 200 mgm. dose is a toxic dose, and blood samples taken during the low calcium tetanic seizures show greatly prolonged clotting times, at times in excess of one hour. The entire picture is similar to that of the oxalate mechanism.

Results of in vitro experimentation. The experiments with fluoride and toxic doses of oxalate lead us to assume a calcium mechanism. *In vitro* experiments show exactly the same results as *in vivo* experiments. The addition of 0.2 cc. of 0.001 molar sodium oxalate to 1.8 cc. of blood reduces coagulation times by 5 to 60 per cent; while 0.2 cc. of 0.1 molar sodium oxalate prevents blood from clotting. The addition of 0.2 cc. of a saturated $CaCl_2$ solution to 1.8 cc. of blood prevents clotting or prolongs it to in excess of 30 minutes. The addition of traces of calcium ion to blood slightly hastens clotting.

Action of dicarboxylic acids on heparinized blood. If it is true that dicarboxylic acids act on the first phase of coagulation, it follows that dicarboxylic acids should have no effect on coagulation time in heparinized

animals. Heparin is known to have an anti-thrombase action which inhibits the second phase of blood coagulation. Rabbits weighing 2.5 kgm. were given 5 mgm. of heparin intravenously after their normal clotting times had been determined. The clotting time was redetermined five minutes after the heparin was injected. Simultaneously, oxalic acid (10 mgm.) was injected intravenously, and 15 minutes later the clotting time was redetermined. The results are summarized in table 3.

It is apparent that the dicarboxylic acids had no significant effect on the clotting time of heparinized blood. This fact points to the first phase of coagulation as the one affected by dicarboxylic acids.

DISCUSSION. All of these facts taken in conjunction point to the existence of a calcium prothrombin or similar calcium combination. With

TABLE 3

The effect of oxalic acid on the coagulation time of heparinized animals

RABBIT, 2.5 KGM.	CLOTTING TIME BEFORE HEPARIN	CLOTTING TIME 5 MIN. AFTER INJECTION OF 5 MGM. HEPARIN	CLOTTING TIME 15 MIN. AFTER INJECTION OF 10 MGM. OXALIC	PER CENT CHANGE IN CLOTTING TIME EFFECTED BY THE ADMINISTRATION OF OXALIC ACID
				per cent
1	5′ 30″	60′	25′	41.5
2	3′ 25″	85′	35′	41.1
3	3′ 23″	40′	20′	50.0
4	3′ 11″	26′	11′	42.0
Heparinized but not treated with oxalic acid				
5	4′ 5″	85′	37′	43.5
6	3′ 21″	67′	28′	41.8

this one assumption, based on the established existence of calcium proteinate combinations, the entire picture becomes harmonious.

The addition of small amounts of oxalate to blood *in vitro* and the intravenous administration of oxalate reduce coagulation time, due to the withdrawal by the oxalate of calcium ion from the blood. Then a simple application of apparent solubility product and mass action law shows that the calcium prothrombin union is broken up, freeing the prothrombin for its subsequent conversion to thrombin. This prothrombin-to-thrombin reaction is catalyzed or facilitated by the presence of calcium ions, and if enough oxalate is added to remove the calcium ion, greatly delayed clotting or no clotting is the result. The dissociation constant and apparent solubility product of the calcium prothrombin combination are smaller and larger respectively than the corresponding values for the calcium oxalate compound, which means that complete dissociation of the calcium prothrombin combination would occur before

the oxalate removed the calcium ion from the blood sample. Thus, the addition of small amounts of oxalate increases the amount of prothrombin free and available for conversion to thrombin; but it does not remove the calcium ions, and hence clotting is hastened. Conversely, the addition of excess calcium by mass action effect forces all of the prothrombin into combination and leaves none in a free condition for conversion to thrombin. Hence, inhibition of the clotting of blood.

The following chart illustrates in diagrammatic form the reactions involved:

Outline of Reactions Involved in the Oxalate Mechanism

$$\text{Calcium Prothrombin} \leftrightharpoons Ca^{++} + \text{Prothrombin} + \text{Oxalate}^{=} \leftrightharpoons \text{Calcium Oxalate}$$

$$\downarrow Ca^{++}$$

$$\text{Thrombin} + \text{Fibrinogen}$$

$$\searrow$$

$$\text{Fibrin}$$

Cephalin combines with and removes the anti-thrombin, heparin.

1. Add calcium ion in excess:

$$\text{Result: } \frac{(Ca^{++})\ (\text{Prothrombin})}{(\text{Ca Prothrombin})} > K$$

Therefore calcium ion and prothrombin combine to form calcium prothrombin until

$$\frac{(Ca^{++})\ (\text{Prothrombin})}{(\text{Ca Prothrombin})} = K$$

Leaving a minimum of free prothrombin for conversion to thrombin, and clotting times are prolonged.

2. Add small amount of oxalate ion:

$$\text{Result: } \frac{(Ca^{++})\ (\text{Oxalate}^{=})}{(\text{Ca Oxalate})} > K$$

Therefore calcium and oxalate ions combine to form calcium oxalate with the consequent withdrawal of calcium ions causing

$$\frac{(Ca^{++})\ (\text{Prothrombin})}{(\text{Ca Prothrombin})} < K$$

Therefore calcium prothrombin dissociates into calcium and prothrombin, liberating prothrombin for conversion to thrombin and hastening coagulation.

3. Add large amount of oxalate:

$$\text{Result: } \frac{(Ca^{++})\ (\text{Oxalate}^{=})}{(\text{Ca Oxalate})} > K$$

Therefore calcium and oxalate ions combine to form calcium oxalate with the resultant removal of nearly all of the calcium ion. This results in complete dissociation of the calcium prothrombin combination, but the calcium ions concentration is too low to catalize the conversion of prothrombin to thrombin reaction.

Mathematically, it is possible to calculate the calcium ion concentrations if certain assumptions are made. The volume of blood in a 2.5 kgm. rabbit must be assumed to be 250 cc. The calcium ion concentration must be assumed to be 5 mgm. per 100 cc. Finally, the oxalate ion concentration must be assumed to be 4 mgm. per 100 cc. From these values the apparent solubility product is calculated to be (4.8) (10^{-7}). Using this value for the apparent solubility product of calcium oxalate, we find the calcium ion concentration of the rabbit's blood following the administration of 60 mgm. dose of sodium oxalate to be (2) (10^{-4}) moles per liter. This is a value of calcium ion concentration which results in an inhibition of the clotting processes. The same value following a 40 mgm. dose of sodium oxalate is (4) (10^{-4}). Thus at an approximate calcium ion concentration of (2) (10^{-4}) moles per liter, coagulation is inhibited, while at a calcium ion concentration of (4) (10^{-4}) moles per liter coagulation is hastened.

It is impossible to calculate similar values for any other acid ion used as no values are known from which the apparent solubility products can be calculated. It is to be emphasized that we apply the law of mass action only to the reactions designated and not to the entire process of clotting. Moreover, we realize that we are measuring speed of a reaction and therefore cannot directly apply the law of mass action to the entire process; but inasmuch as calcium ion concentration and free prothrombin concentration are known to control the speed of the reaction, we feel justified in applying the law of mass action indirectly to the entire process, as we apply it directly to the reactions listed.

The study of the action of dicarboxylic acids in normal animals leads us to suggest a similar explanation for the effects observed in hypo-prothrombinemia due to a lack of the anti-hemorrhagic vitamin. A calcium prothrombin combination exists in the blood. For its conversion to thrombin, the prothrombin must be liberated from the combination. This is the action of the dicarboxylic acid. The mobilization of the prothrombin results in the decreased clotting times observed by us.

SUMMARY

Dicarboxylic acids reduce coagulation time in vitamin K deficient chicks but do not alter coagulation time in heparinized animals. Fluorides reduce coagulation times in normal animals. Evidence is presented indicating that the action of fluorides and dicarboxylic acids is one determined by the law of mass action involving directly calcium ion concentrations and indirectly prothrombin concentration. A calcium prothrombinate is hypothesized.

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THE EFFECTS OF PHLORHIZIN ON RENAL PLASMA FLOW, ON GLOMERULAR FILTRATION AND ON THE TUBULAR EXCRETION OF DIODRAST IN THE DOG[1]

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The action of phlorhizin in suppressing the tubular reabsorption of glucose is well known; knowledge of its action on transfers from blood into tubular lumen is less complete. Under phlorhizin the normal excess of exogenous creatinine over inulin clearance in man (1), the apes (2), the chicken (3), and the teleost (4) and elasmobranch (5) fishes is abolished or reduced; this has been interpreted as meaning that it abolishes or reduces a preëxisting tubular excretion of creatinine. Similar evidence has been cited (6) for a depression of the tubular excretion of creatine by phlorhizin in the marine teleost, *E. morio*, although the tubular excretory mechanism for creatine in the dogfish is reported as not being affected by phlorhizin except where renal circulation is severely depressed (7). Phlorhizin has no effect on the tubular excretion of phenol red in the dog (8), except for transitory depression which appears to be due to circulatory changes. In the chicken phlorhizin depresses the tubular as well as the glomerular excretion of phenol red, "but whether this is a result of a reduction of blood flow to the kidney or whether the tubular mechanism by which phenol red is excreted is specifically affected, cannot be determined from the present data" (9). The question of a direct action of phlorhizin on tubular excretory mechanisms appears to be unsettled except in the case of creatinine, where evidence for such action is strongest.

Difficulties of interpretation have been increased by the circumstance that in most of the above cited work doses of phlorhizin designed to block completely the reabsorption of glucose have been used. Intravenous administration was usually employed, alone or supplemented by subcutaneous administration; circulatory disturbances are often produced, sometimes with nausea and vomiting in mammals. Such disturbances are negligible in dogs if a dose of not more than 0.2 gram per kilo is given subcutaneously and 1.5 hours allowed to elapse before clearance observations are begun. Such a procedure may or may not completely phlor-

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hizinize the dog, where identity of glucose with inulin or creatinine clearance is the criterion of completeness. In any event the glucose clearance will be 80 per cent or more of the inulin. This effect is adequate for our purpose, which is to see whether a dose of phlorhizin adequate to bring on marked if not complete suppression of glucose reabsorption, with little or no circulatory effect, will affect tubular excretion of diodrast (D). As a further check on what is happening to the renal circulation, beyond that afforded by the behavior of the absolute values of inulin and D clearances, renal plasma flow (RPF) was determined in two experiments on two renal explant dogs by following the extractions of inulin and D. A depression of the tubular excretion of D by phlorhizin was found, over and above any effect due to circulatory changes.

METHODS. Inulin, glucose and D plasma clearances were carried out either at constantly maintained plasma levels throughout the experiment, or with falling plasma levels of inulin and D following two urine periods within which a constant and high level of D was maintained. Four phlorhizin experiments with 18 urine periods have been carried out on 4 dogs; many more control or nonphlorhizin periods were obtained on these dogs. In 3 experiments 0.2 gram of phlorhizin per kilo, as a 10 per cent solution in 2 to 2.5 per cent sodium bicarbonate solution, was given subcutaneously 1.5 hours before the first blood collection; in one experiment 0.1 gram per kilo was given intravenously over a 12 minute period, with the next blood collection 17 minutes after the end of the phlorhizin injection. Maximum rates of tubular excretion of D (Tm) have been followed, as well as clearances at lower plasma D levels. Trained, unanesthetized female dogs were used; inulin (10) and D (11) were given by intravenous infusion; a Shaffer-Somogyi copper reagent was used in glucose determinations. Urine inulin readings were corrected for glucose contribution to color; glucose effect on plasma inulin readings was compensated by using a blank filtrate (before inulin administration) for the zero setting in the colorimeter. This precludes the necessity of glucose removal by fermentation, since our plasma glucose levels remained very constant, with variations of less than 5 mgm. per cent throughout an experiment.

RESULTS. *Diodrast plasma clearance.* At all plasma D levels investigated the D plasma clearance under phlorhizin is considerably below the normal for the corresponding plasma D level. Figure 1 shows normal clearances on 4 dogs as solid circles and clearances under phlorhizin as open circles on the same dogs. The percentage reduction at low plasma D levels is somewhat greater than at high levels; this is explained by the following circumstances. The higher the plasma level above that at which self-depression of D clearance begins (about 13 mgm. I per 100 cc.), the greater is the percentage of D excretion contributed by the glomeruli.

At a plasma level of 30 mgm. I per 100 cc. the excretion of D in normal dogs is equally divided between glomeruli and tubules and as the level rises the glomerular contribution rises above 50 per cent. If phlorhizin depresses only the tubular contribution, it follows that its effect on total clearance will be less at high plasma levels, where the glomerular contribution becomes more and more important. One would thus predict from an inspection of figure 1 that glomerular filtration rate (inulin clearance) is less affected by phlorhizin in these experiments than is tubular excretion of D, and this is found to be true.

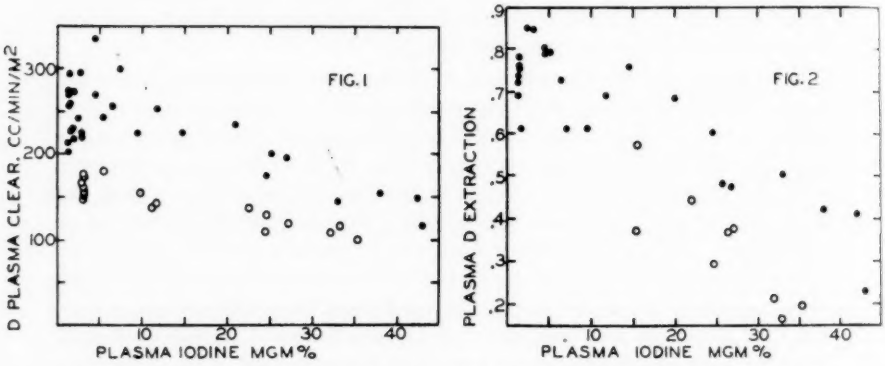

Fig. 1. Comparison of normal D plasma clearances (solid circles) with D plasma clearances under phlorhizin (open circles) at various plasma I levels, I being a measure of D.

Fig. 2. Comparison of plasma D extractions in normal (solid circles) and phlorhizin (open circles) periods at various plasma I levels.

Extraction of diodrast. As would be predicted from the clearance effects, the plasma extraction, $\frac{A - RV}{A}$, of D is lowered by phlorhizin. Figure 2 shows plasma D extractions under phlorhizin as open circles, as compared with extractions without phlorhizin. Renal vein bloods were not obtained in the 2 experiments (7 periods) with phlorhizin at plasma levels of about 3 mgm. I per 100 cc. seen in figure 1.

Renal plasma flow. The preceding 2 sections indicate that the lowered D plasma clearance seen under phlorhizin is due to tubular depression but do not afford unqualified proof of this. This proof can be given by showing that the effects cannot be referred to circulatory changes. If it can be shown that RPF is not lowered and that glomerular filtration rate is not greatly lowered by phlorhizin in these experiments, it follows that the depression of clearance must be due to a specific depression of tubular

excretion by the drug. Renal plasma flows were obtained on 2 dogs, as inulin plasma clearance/plasma inulin extraction and as D blood clearance $\times$ V_p/blood D extraction (12); the 2 methods agree closely and their average is given here. One dog (K1) showed an average normal RPF of 285 cc./minute/M^2 while the phlorhizin periods averaged 307; the other dog (K3) averaged 396 normal and 408 with phlorhizin.

Glomerular filtration rate. The inulin clearance in the 3 dogs where 0.2 gram phlorhizin subcutaneously was given 1.5 hours before the beginning of the clearance observations averaged 94 cc./minute/M^2 in the normal periods and 67 in the phlorhizin periods. The dog (K7) which was given 0.1 gram per kilo intravenously 17 minutes before the next period started averaged 71 cc./minute/M^2 in the normal and 60 in the phlorhizin periods. It thus appears that some fall in glomerular filtration results from phlorhizin as given in these experiments. This fall, however, is quite inadequate to account for the total fall in D plasma clearance, as will be pointed out later. Since it is not accompanied by any fall in RPF, it must be explained by a slight constriction of the afferent vessels, presumably accompanied by a corresponding dilatation distally.

Tubular clearance of diodrast. One can get an expression for tubular clearance of D by dividing tubular output in mgm./minute/M^2 by plasma level; tubular output is total output minus glomerular output, the latter being calculated from filtration rates and filterable fraction of D (13). If the fall in total (glomerular plus tubular) plasma clearance of D under phlorhizin were due solely to the fall in glomerular filtration rate, the tubular clearance would remain unchanged; actually it is considerably reduced. Taking only the periods where plasma I level is below 13 mgm. per 100 cc., i.e., the periods in which there is no self-depression of D clearance, the 4 dogs show an average tubular D clearance of 173 cc./minute/M^2 in the normal and 110 in the phlorhizin periods. The average total plasma clearance of D drops from the normal of 267 cc./minute/M^2 in these experiments to 177 under phlorhizin; of this drop of 90, the fall in glomerular output accounts for only 27. Depression of tubular output by phlorhizin thus accounts for 70 per cent of the drop.

Even though it is shown that the tubular excretion of D is lowered by phlorhizin it is still not proved that any specific disturbance of the excretory mechanism is being produced until it is shown that the fall cannot be explained merely on the basis that less D is being brought to the tubules. The best expression of the tubules' efficiency in excreting D is what may be termed the "tubular extraction" of D, which can be expressed as tubular D plasma clearance/RPF. It designates the fraction of RPF which is cleared of D *by tubular activity.* A fall in this value indicates a fall in the inherent ability of the tubules to excrete D and its value will

not be affected by changes in RPF unless the latter falls so low as to damage the tubules by ischemia, which is not the case in these experiments. This fraction has averaged 0.55 in the normal periods and 0.31 in the phlorhizin periods. It thus appears that the efficiency of the tubules in excreting D at plasma levels below 13 mgm. I per 100 cc. is lowered by phlorhizin in these experiments to about 56 per cent of the normal and that the depressant effect cannot be explained on the basis of a tubular ischemia.

Effect on maximum tubular excretory rate. Observations on the maximum rate of tubular excretion (Tm) of D as affected by phlorhizin have been made on 2 dogs. In these dogs D Tm was normally attained at a plasma level of 18 to 20 mgm. I per 100 cc. and averaged 24.3 mgm. I/minute/M^2; under phlorhizin the plasma level necessary to bring on D Tm was the same as in the normal periods (somewhere between 16 and 20 mgm. I per 100 cc.) but the value of D Tm averaged 15.5 mgm./minute/M^2. The maximum ability of the tubules to excrete D under phlorhizin is thus 64 per cent of that without phlorhizin; here also the effect is not due to any fall in RPF. It thus appears that the maximal tubular excretory power for D is depressed by phlorhizin in these experiments to about the same extent as is their efficiency in extracting D at low plasma levels, i.e., to about 60 per cent of the normal; this identity of effects at high and low plasma levels is of some theoretical interest.

Glucose clearance. The tubular reabsorption of glucose was usually not completely abolished by phlorhizin as given here; the ratio glucose/inulin plasma clearance averaged 0.85. With phlorhizin doses and time relations adequate to insure a unity glucose/inulin clearance ratio an even greater depression of tubular excretion of D would probably be obtained. It was considered more desirable, however, to restrict the phlorhizin dose so as to minimize circulatory changes even though glucose reabsorption was not completely suppressed, since our arguments do not demand such completeness.

SUMMARY

A dose of 0.2 gram phlorhizin per kilo subcutaneously given 1.5 hours before beginning observations lowers the plasma clearance and renal extraction of D considerably more than can be accounted for by the slight lowering of glomerular filtration rate which results. Renal plasma flow is unchanged. The ability of the tubules to excrete D is lowered to about 60 per cent of the normal at both high and low plasma D levels. The work was done on unanesthetized dogs.

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THE GREATER RESISTANCE OF VERY YOUNG ANIMALS TO ARREST OF THE BRAIN CIRCULATION[1,2]

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Previous investigations have revealed that the young animal is more resistant to asphyxia than is the adult (1, 2). Recently, it has been demonstrated that the brain itself is more resistant in the young animal, since young dogs were found to be less susceptible than adults to complete arrest of the brain circulation (3). Careful studies of the changes in vascularity in various portions of the rat brain from birth to maturity (4, 5, 6) have revealed a rapid and progressive increase in vascularity during the developmental period. It was therefore deemed advisable to investigate quantitatively the course of the change in resistance to arrest of the brain circulation during the period of rapid growth.

The basis for such a quantitative investigation is the remarkable constancy of the effects produced by a given period of complete cephalic stasis in adult dogs (7, 8). Thus, all normal adult animals studied had recovered completely several weeks after arrest of cephalic blood flow for a period of six minutes or less. On the other hand, severe permanent brain damage was evident in every adult dog subjected to eight minutes of arrest of the brain circulation. The symptoms and the course of recovery of adult dogs subjected to the same period of arrest of blood flow were practically identical regardless of differences in size, age, sex or breed of the experimental animals.

METHOD. The method employed was essentially the same as had been used previously in the investigation of adult dogs (7, 8). A preliminary aseptic operation, involving removal of the spine and laminae of the second cervical vertebra, was performed under ether or pentobarbital a day or two before the experiment.

A day or two after operation, following insertion of a metal tracheal tube through the glottis and administration of atropine, a cervical pressure cuff

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[2] Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration, Official Project no. 665-71-3-69, sub-project no. 309.

was inflated suddenly by means of compressed air to a pressure of 600 to 800 mm. of mercury. In this way, the blood flow to the brain was stopped suddenly and completely. Artificial respiration was given when the respiratory center began to fail, and was continued during and following the arrest of circulation until respiratory function was again established. The effects of arrest of the cephalic circulation have been studied in 42 dogs, ranging in age from 8 to 141 days. The animals varied in weight, sex and breed. Littermates were studied using different periods of brain stasis.

The argument that the high pressure over the spinal cord in the region of the laminectomy may cause damage to the spinal cord and thus complicate the results has been discussed elsewhere (8). It will be evident from the results to be presented that this is not a significant factor.

RESULTS. *A. Quantitative study of revival time of brain function.* The maximal duration of arrest of the cephalic circulation from which brain function can recover completely has been studied in dogs at different ages. This "revival time" (9) decreases progressively as the animal grows older. This study is illustrated graphically in figure 1. All points on or below the lower curve (absolute revival time) represent animals which recovered completely in a relatively short time following arrest of cephalic blood flow and lived a normal existence for months afterwards. This curve, then, represents the duration of cephalic stasis from which animals can undoubtedly recover completely. It is, however, questionable whether these figures represent the maximal period of cephalic stasis compatible with complete recovery. One needs only to recall that adult dogs, following a six-minute period of cephalic stasis, remain in coma for 24 to 36 hours and show neurological symptoms such as ataxia for several weeks, after which recovery is apparently complete (8). While the animals represented in figure 1 by open circles had neurological symptoms until they died, most of them only lived for 1½ to 10 days following arrest of the cephalic circulation. The symptoms which they showed were relatively mild in most instances. These animals were able to stand and walk, to lap milk, swallow and sometimes nurse on the day following the arrest of cephalic blood flow. Their symptoms consisted of ataxia, tremor and lack of spontaneous activity. Furthermore, many of these young animals showed improvement during the few days that they remained alive, and it is not excluded and even seems probable that recovery would have been complete had they survived for a longer period. The animals died either of secondary upper respiratory or gastrointestinal infection or of poor nutrition and dehydration. When one considers the difficulties involved in feeding and caring for these young nursing animals, such results are not at all surprising. It is necessary, therefore, to revise our estimates of revival time upward and the upper curve in figure 1 seems likely to be closer to the correct figures.

The change in resistance to arrest of the cephalic circulation with age

is thus well shown by the study of revival time. At every age, there appears to be a critical revival time beyond which complete recovery is impossible, and this is as true at 8 to 10 days of age as it is in the adult. While the adult has a revival time of 6 minutes, at 79 days the revival time is 10 minutes, at 48 days 12 minutes, at 24 days 14 or 15 minutes, at 10

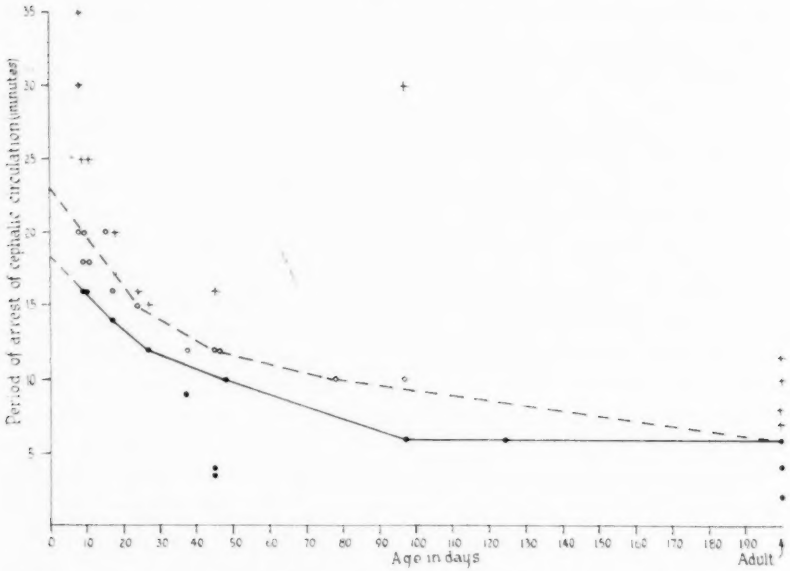

Fig. 1. Maximum period of cephalic stasis compatible with complete recovery of brain function at various ages (revival time). The solid circles represent animals which showed complete recovery of brain function and lived for months after cephalic stasis. The solid line indicates the absolute revival time. The open circles represent animals which had some symptoms of brain damage following cephalic stasis and remained alive for 1½ to 10 days. The dotted line indicates the possible or probable revival time. The crosses represent animals which showed evidence of severe brain damage and died as a rule within 24 hours. Further explanation will be found in the text.

days close to 20 minutes. At birth, continuing this trend, one might expect a revival time of approximately 23 minutes.

The decrease in resistance of the brain to stasis with increasing age of the developing animal was also brought out clearly by the study of littermates at different ages. Two littermates, A and B, males of the same size, weight and color, were subjected to arrest of the cephalic circulation, A at the age of 27 days and B at the age of 124 days. On the day following a 12-minute period of arrest of cephalic blood flow, A was awake and could

stand and walk but fell frequently as a result of ataxia. At 48 hours the animal was entirely normal except for a slight ataxia which soon disappeared. B at the age of 124 days was subjected to a period of cephalic stasis of six minutes, only half the period of stasis to which the littermate had been subjected some three months earlier. For the first 48 hours after arrest of blood flow of six minutes' duration, B was unconscious or had only very dim consciousness of his surroundings. He was unable to turn from his side to his abdomen, stand or walk, and lay on his side showing no spontaneous movements. It was not until the fifth day that he regained full consciousness, could turn from his side to his abdomen and sit up. He was still unable, at this stage, to stand or walk. At the end of a week, he was alert and curious and was able to stand and walk but fell very frequently due to the severe ataxia. The ataxic symptoms gradually improved, and disappeared a month after the cephalic stasis. Both A and B are still alive at this writing, $8\frac{1}{2}$ months and $5\frac{1}{2}$ months, respectively, after the arrest of blood flow, and neither animal can be distinguished from the normal. The effects of a 6-minute period of arrest of brain circulation were thus more severe at 124 days of age than a 12-minute period of circulatory arrest in a littermate 27 days of age.

A similar experiment was performed in which two littermates were subjected to arrest of cephalic circulation of ten minutes' duration at different ages. Dog 9, following 10 minutes of arrest of blood flow at the age of 48 days, was able to stand and walk ataxically after three hours. After six hours, he was alert, curious and responsive, came when he was called, fed himself and was quite normal except for a slight ataxia, and after 24 hours had recovered completely. On the other hand, dog 10, at the age of 77 days, had much more severe symptoms following 10 minutes of cephalic stasis. On the day after the arrest of circulation, the animal lay on his side in coma, motionless and unable to turn from the side to the abdomen. By the fourth day, he had recovered consciousness and could sit up and eat but was unable to stand or walk. On the fifth day he was more curious, active and responsive, and could stand and walk, although the very severe ataxia resulted in frequent falling. Thus, dog 10, although only a month older than his littermate, was severely affected by the same period of cephalic stasis from which the littermate had recovered in a few hours.

B. Loss of function during complete arrest of the cephalic circulation. The time during which various functions persist, despite complete cessation of blood flow in the brain, is an index of the resistance of the neural structures to anoxia which has been designated the "survival time" (9). Studies of the corneal reflex, respiration and spontaneous somatic movements in dogs at various ages show that the survival of reflexes of animals 8 to 11 days of age is very much longer than in adult dogs. The index of

resistance to arrest of cephalic blood flow (adult = 1), derived from the data on respiratory survival time, provides a rough estimate of the quantitative change in resistance with age. When the index of resistance of the respiratory center is plotted against age, one obtains a hyperbolic curve (fig. 2), indicating that the decline in resistance to anoxia is very rapid during the first post-natal month and slows down progressively in the second, third, and fourth months of life. An animal at four months of age is as susceptible to cephalic stasis as the adult dog. When the curve is extrapolated to zero age (birth),[3] the resistance of the respiratory center to arrest of its circulation appears to be approximately seventeen times that of the adult. The respiratory survival time during brain stasis is 20–30 seconds in the adult dog and five minutes in dogs 8 to 10 days of

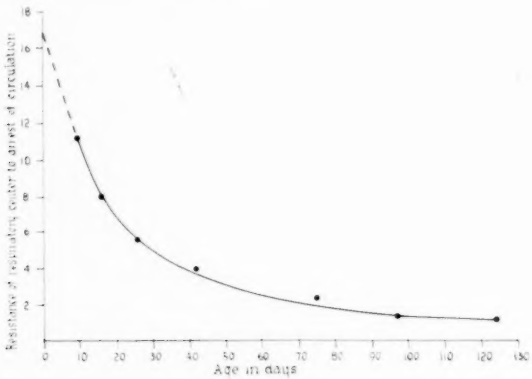

Fig. 2. Influence of age on the resistance of the respiratory center to arrest of circulation. The resistance of the respiratory center represents the index of relative resistance based on survival time, with adult = 1.

age. Respiratory efforts persist for 27 minutes during asphyxia in dogs 1 day old, while they cease in 2 to 4 minutes (10) in asphyxia in the adult.

During arrest of cephalic blood flow in the adult dog, after cessation of respiratory reflexes and of spontaneous somatic movements, a state of profound spinal shock becomes evident (8). This is readily made apparent by disappearance of the flexion reflex to noxious stimulation of the foot. The spinal shock often persists for some time after arrest of blood flow and spinal reflex irritability is only gradually restored. In young dogs, on the other hand, there was no evidence of spinal shock during complete arrest of blood flow in the brain. In eleven animals, 8 to 11 days of age, the flexion reflex could be elicited readily throughout the period of cephalic

[3] The validity of such extrapolation may be open to question.

stasis, even though the stasis was continued for as long as 35 minutes. The flexion reflex was also present throughout the period of arrest of cephalic circulation in dogs 17, 24, and 27 days of age. Evidence of mild spinal shock was found in experiments on dogs 45 days of age. In a dog 97 days of age subjected to complete arrest of brain circulation for a period of 6 minutes, the flexion reflex could no longer be evoked after 2 minutes, 50 seconds of stasis, and the reflex did not reappear until one minute after restoration of blood flow. At four months of age, spinal shock appeared during cephalic stasis and was only gradually dissipated afterwards. At first, after restoration of blood flow in the brain, only the flexion reflex was elicited and the crossed extension reflex did not reappear until several minutes later.

C. Immediate return of function after restoration of cephalic blood flow. After the blood was allowed once more to flow through the brain, this organ gradually recovered its functions. These functions were restored in the order opposite to their disappearance during cephalic stasis. The first sign of recovery was movement of the tail or movements of the skin followed by movements of the limbs. Soon after this, the first inspiration occurred, a strong inspiratory movement accompanied by opening of the mouth, repeated irregularly and infrequently. Some time after the beginning of respiratory efforts, the corneal reflex was restored and after another short interval, respiration became normal in rhythm and frequency.

The speed of recovery of the lower centers involved in these functions depends on two factors: the duration of arrest of the brain circulation, and the age of the animal. The quantitative results on "recovery time" of respiration (appearance of the first inspiration) and of the corneal reflex in dogs at various ages is illustrated in figure 3. Most of the points represent the average recovery time of a number of animals. Restoration of function of the corneal reflex is greatly delayed compared to the respiratory center, being on the average about four times that of respiration. For any period of cephalic stasis, the younger the animal, the shorter the recovery time and, with increasing age, the recovery time of the young animal approaches that of the adult dog. In general, regardless of age or the duration of cephalic stasis, when the respiration is revived within $2\frac{1}{2}$ minutes and the corneal reflex within 12 minutes, later recovery of brain function is apparently complete. On the other hand, longer recovery times beyond these critical values result in more or less severe permanent damage of the brain. (One must keep in mind that these are average figures and must allow for individual variations.) The average critical figures for recovery time are somewhat lower for the adult dog, being two minutes for respiration and less than eight minutes for the corneal reflex.

D. Asphyxia in the neonatal period. Anoxia was produced in 11 dogs and 6 rats in the neonatal period by causing the animal to rebreathe a

small volume of pure nitrogen. In the dogs, a small metal cone was employed, the open end of which was covered with a rubber membrane containing an opening just large enough to admit the head. This cone was filled with nitrogen by blowing a stream of the gas through it for a minute or two, following which the cone was placed in position. The stretched rubber membrane fitted around the neck and was air-tight. The rats were placed in a short wide test tube full of nitrogen which was stoppered.

The results are presented in table 1. It is interesting to compare these results with the effects of asphyxia in the adult dog. According to

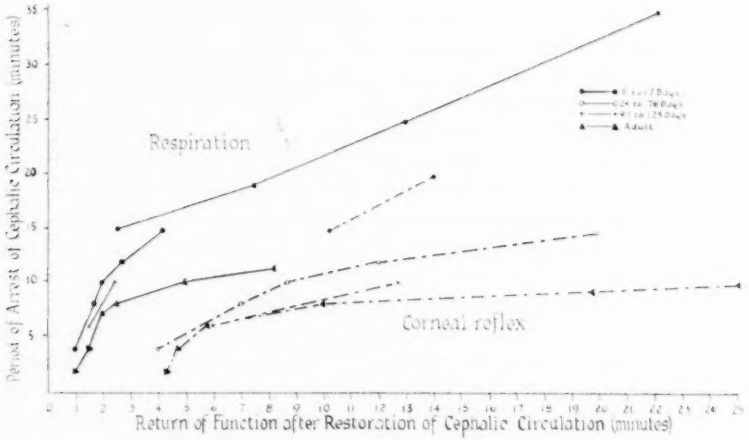
Period of Arrest of Cephalic Circulation (minutes)
Respiration
Corneal reflex
Return of Function after Restoration of Cephalic Circulation (minutes)

Fig. 3. Recovery time of respiration and the corneal reflex in dogs at various ages. The solid lines represent recovery time of respiration (first gasp); the broken lines represent recovery time of the corneal reflex. The symbols for the various age groups are indicated in the figure. Most of the points represent the average recovery time of a number of animals. The data for the corneal reflex in the youngest age group were very meager because most of these animals did not yet have the eyes open.

Lougheed, Janes and Hall (10), adult dogs never recover after seven minutes of acute asphyxia, rarely recover after four minutes, and in some cases do not recover after only two minutes of asphyxia. In all of the dogs one day of age which died (1, 4 and 5), inspiratory movements returned during the first minute of resuscitation but persisted only 4 to 5 minutes and then stopped. Soon after cessation of respiration, the flexion reflex disappeared. It was then found impossible to resuscitate the animal, despite prolonged and efficient artificial respiration. In the two other experiments which terminated fatally, no respiratory effort was made following renewal of the oxygen supply.

All of the young dogs and rats which survived the severe acute asphyxia recovered function very rapidly. The animal which recovered from anoxia of 25 minutes' duration sat up and moved 11 minutes later and became active and began to nurse 17 minutes after the end of anoxia. After 20 minutes of anoxia, the newborn animal, though unable to turn from the

TABLE 1

Acute anoxia in the neonatal period

		LOSS OF FUNCTION				RETURN OF FUNCTION		
DOG	AGE	Duration of anoxia	Respiration stops	Movements cease	Flexion reflex	First inspiration	Flexion reflex	Result
	days	*min.*	*min.*	*min.*				
1	1	19	18	18	18 min. 25 sec., weak; 18 min. 40 sec., gone	50 sec.	1 min., good	Respiration stopped at 4 min., died
2	1	20	16	8	13 min., weak; 16 min., gone (pinch foot causes gasping)	4 min.	Returns	Lived
3	1	25	23	20	Good throughout	20 sec.	Never lost	Lived
4	1	35	34	17	Good throughout, weaker at end	45 sec.	30 sec., good; 2 min. 30 sec., gone; 3 min., weak	Respiration stopped at 5 min. 45 sec., died
5	1	41	40	30	40 min. 30 sec., weak	1 min.	30 sec., weak	Respiration stopped at 4 min. 40 sec., died
6	2	15	14			None		Died
7	2	17½	14			None		Died
8	3	16	14			4 min.		Lived
9	3	17	16			3 min.		Lived
10	9	7	Continues			Never lost		Lived
11	9	17	16	6		3 min.		Lived
RAT								
1	1½	30	Continues	Continues		Never lost		Lived
2	1½	40	Continues	Stopped		Never lost		Lived
3	1½	44	Continues	Stopped		Never lost		Lived
4	1½	45	Continues	Continues		Never lost		Lived
5	1½	61½	54	32		30 sec.		Lived
6	1½	70½	56	45		30 sec.		Lived

side to the abdomen 24 minutes later, was moving ataxically 32 minutes after the readmission of oxygen. On the day following the experiment, the animals could not be distinguished in any way from control animals. Moreover, some of the dogs and all of the rats were observed for a number of weeks, and their growth, activity, neurological status and apparent cerebral function were all unimpaired by the prolonged asphyxia. Acute

complete anoxia in the newborn would thus appear to be an "all-or-none" reaction, resulting in either complete recovery or an immediate fatal termination. This would seem to indicate that, as in the adult (10, 11), the heart is more sensitive to asphyxia than is the brain.

Discussion. An unexpected result of this investigation was the observation that spinal shock does not appear in very young animals during arrest of the brain circulation as it does in adult animals. A survey of the available literature has failed to reveal a previous report of this finding. Since the degree of spinal shock which is produced in animals is less the lower the species in the evolutionary scale, this is, perhaps, another example of ontogeny recapitulating phylogeny. Viewed in this light, one may, perhaps, consider the generally greater resistance of newborn animals to anoxia also as an example of this fundamental biological principle, since lower forms of animals are known to be more resistant to anoxia than higher forms.

Evidence has been presented that the brain of the very young animal is very resistant to arrest of its circulation and that this resistance decreases rapidly at first and then more slowly with advancing age. The greater resistance of the young brain has been demonstrated by studies of loss of function during brain stasis (survival time), recovery of function after restoration of cephalic circulation (recovery time) as well as the ability to recover completely following prolonged arrest of circulation (revival time). A greater resistance to anoxia of the heart, as well as the brain, has also been indicated by the long survival and revival times of acute complete anoxia in the newborn. All of these indices of resistance to anoxia were consistent in illustrating the factor of age. The change in resistance to anoxia from birth to maturity is of a magnitude beyond the probable error involved in these experiments, since the resistance of the newborn as shown by revival time was 400 per cent greater than the adult, while the resistance of the newborn respiratory center as shown by survival time was perhaps 1700 per cent greater than the adult.

Previous investigators have established the fact that newborn animals are more resistant to anoxia than are adults. Reiss and Haurowitz (1), who limited their study to survival time, found that newborn mice were more resistant than adult mice, whether the anoxia was produced by carbon monoxide, hydrogen, carbon dioxide or hydrogen cyanide, while age was not a factor in susceptibility to chloroform. Calculating from their data, the ratio of resistance of newborn to adult for pure carbon monoxide is 132:1 and for hydrogen cyanide 117:1. Avery and Johlin (2) also demonstrated the greater resistance of young animals to carbon monoxide asphyxia. In the course of other studies, incidental observations have been made on the greater resistance of the young brain to arrest of its circulation by Crile and Dolley (12) and Andreyev (13).

The greater resistance of the brain of the newborn to arrest of circulation is probably dependent on metabolic factors. The basal metabolism of the newborn per unit weight is greater than that of the adult, but per unit body surface is smaller than in the adult (14). Himwich and his co-workers (15, 16) have studied the metabolism of infant brain slices, in vitro, and found that the QO_2 per unit dry weight was the same as that of the adult in the absence of substrate or in the presence of lactate. On the other hand, in terms of dry weight, in the presence of glucose the QO_2 of the brain of infants 1 to 11 days of age was 11.7, while at 12 to 24 days of age it was 13.6, which is the adult level. Per unit *wet* weight of minced brain, the O_2 uptake of the infant was 123 compared with 200 for the adult. This is a much smaller difference than has been noted in respiratory survival time and in revival time of very young and adult dogs in our experiments. The difference in rate of oxygen utilization of the brain of the infant and the adult may thus be a minor factor in the greater resistance to anoxia of the infant brain.

Another factor to be considered in relation to the resistance of the brain to anoxia is the ability of the neurons to obtain energy in the absence of oxygen. Reiss (17) reported that the major factor involved in the resistance of newborn mice and rats to anoxia (carbon monoxide poisoning) was the ability to break down carbohydrate to lactic acid anaerobically. He compared the lactic acid content of the entire body of normal and fatally asphyxiated animals in the newborn and the adult. The increase of lactic acid produced by asphyxia in the newborn was 460 per cent compared to an increase of lactic acid of 23 per cent in asphyxia in the adult animal. Reiss also noted that death occurred from asphyxia in the newborn with a large carbohydrate reserve still present. Moreover, while the animals succumbed much more rapidly to anoxia at 37°C. than at room temperature, the lactic acid levels were the same at the time of death. This indicates that the cause of death may be accumulation of lactic acid. Since the increased resistance to anoxia in the newborn appears to involve the heart and other tissues as well as the brain, it is a reasonable assumption that the infant brain shares in the increased anaerobic glycolysis.

Reiss' (17) figures for lactic acid and our own data for revival time correspond closely. Reiss found that the increase of lactic acid production in asphyxia in the newborn as compared to the adult was 430 per cent. The data presented above on revival time (fig. 1) indicate that the brain of the newborn is approximately 400 per cent more resistant than that of the adult to arrest of circulation. It is interesting to note that Craigie (5) found an increase in vascularity of the rat parietal cortex from birth to maturity of 440 per cent.

Statements appear in the literature without experimental evidence

that the brain of the newborn is *less* resistant to anoxia than the adult brain. Thus, in a discussion of paranatal asphyxia, Schreiber (18) states: "Considerable clinical evidence supports the conclusion that the brain tissue of an infant can sustain much less oxygen deprivation than can the adult organ, and therefore, is more readily damaged from this particular cause than is adult cerebral tissue." Clinical observations on asphyxia neonatorum (19) reveal, however, that the newborn infant can recover completely after severe asphyxia prolonged far beyond what would constitute fatal asphyxia in the adult. The great resistance of the respiratory center of the newborn is attested to by the prompt response of severely asphyxiated infants to lobeline. There seems to be good reason to believe, therefore, that the brain of the human infant is more resistant to anoxia than the adult organ.

SUMMARY

1. The young dog is much more resistant to acute asphyxia than is the adult animal.

2. The brain of the young animal is much more resistant to arrest of its circulation than is the adult organ. This has been demonstrated by quantitative studies of survival time, recovery time and revival time after temporary complete arrest of the cephalic circulation.

3. In the newborn, the respiratory center will apparently continue to function 17 times as long as in the adult during complete arrest of blood flow in the brain.

4. The ability of the newborn to achieve complete functional recovery following periods of complete arrest of the brain circulation is approximately 400 per cent greater than that of the adult.

5. The change with age in resistance of the brain to arrest of its circulation follows a hyperbolic curve, decreasing rapidly in the first month and more gradually later. The resistance is diminished to the adult level at the age of four months.

6. Spinal shock does not supervene during arrest of the brain circulation in young dogs.

7. The relation of the increased resistance to brain anoxia in young animals to changes with age in metabolism and vascularity of the brain is discussed.

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HORMONAL INFLUENCES ON CARBOHYDRATE METABOLISM DURING WORK

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The effect of adrenalin on the carbohydrate metabolism during muscular exercise has been studied by Dill, Edwards and de Meio (1935), who found that the RQ after adrenalin injections was considerably higher than in control experiments without adrenalin. They concluded that with the doses of adrenalin used the utilization of sugar was increased. This conclusion has been questioned by Courtice, Douglas and Priestley (1939), who assert that the initial increase in RQ depends on the increase in blood lactic acid rather than on oxidative changes and that a subsequent period with subnormal RQ when lactic acid is being removed brings the RQ for the whole period of accumulation and disappearance of lactic acid to the level of control experiments without adrenalin. As the material published by Courtice, Douglas and Priestley does not, in our opinion, convincingly support this assertion, we have repeated the experiments under standardized conditions and with special care for the control experiments.

The procedure followed is very close to that followed by Courtice, Douglas and Priestley. The work consisted in walking on the treadmill at 5.6 km. an hour at a grade of 8.6 per cent for three hours. Expired air was collected for ten out of every fifteen minutes during the experiments, except during the 30 minutes following the injections when collections were made for four minutes out of every five. Blood samples, venous or capillary, for determination of blood sugar, lactic acid, and ketone bodies, were drawn regularly at thirty-minute intervals, but, whenever necessary, at shorter intervals. Urine was voided before work, just after work, and one-half hour later, and was tested for acetone.

The subjects (A, 32 years, and W, 26 years) came fasting to the laboratory in the morning. They lived on an ordinary mixed diet. Subject W did not change this diet throughout the period of experiments while subject A, about halfway through the period, changed his diet to one considerably richer in carbohydrates. The injection of 1 mgm. of adrenalin chloride (Parke, Davis & Co., 1:1000) was made intramuscularly at the end of the first hour of walking, the first hour of work being used as a control. In further experiments 3.5 units of insulin (solution of zinc-insulin

crystals, Lilly) was given intravenously, either alone or combined with adrenalin chloride intramuscularly or sugar orally.

The air samples were analyzed at the Haldane apparatus, blood sugar determined after Folin and Malmros (1929), lactic acid after Edwards (1938), and blood acetone after Van Slyke and Fitz (1917). One week or more elapsed between performances in order to secure complete recovery.

Control experiments. Control experiments showed that the effects of the work on metabolism, RQ, and blood compounds were very well reproducible as long as the diet was unchanged; in subject A, when at the time of the last experiments a carbohydrate-rich diet was being taken, the only effect found was a slightly higher trend in the RQ than in the first experiments. This effect of the diet on the RQ has been shown recently by Christensen and Hansen (1939), but it is emphasized that the RQ in exercise may be altered by other factors than diet. Unpublished experiments by Dill show that even on a prolonged constant diet considerable changes in the trend of the RQ during constant work may be found, and we had, in

TABLE 1

SUBJECT	CALORIES				CALCULATED RQ PER HOUR			TOTAL CARBOHYDRATE	CALORIES DERIVED FROM CARBOHYDRATE
	1. hour	2. hour	3. hour	Total	1. hour	2. hour	3. hour		
								gm.	*per cent*
W	498	492	507	1,497	0.881	0.833	0.806	170	45.6
AI	536	536	547	1,619	0.884	0.844	0.807	191	47.1
AII	534	542	558	1,634	0.900	0.877	0.837	229	55.9

connection with the present experiments, to give up a third subject, a young negro, because his RQ during work showed large differences from week to week, although his diet was as constant as that of A and W. Subjects A and W showed during the three hours of walking a steadily increasing O_2 intake. In the case of A it rose from about 1.8 l/min. at the start to about 1.9 l/min., and in the case of W from about 1.6 l/min. to about 1.7 l/min. The RQ decreased during the same time from about 0.91 at the start to about 0.80 at end of the work. Neither blood sugar nor lactic acid nor blood acetone showed any considerable changes during the work. (See figs. 1–5.) The total amount of work done and the amount of carbohydrate burned during this work are averaged in table 1.

The carbohydrate burned is calculated from the RQ without regard to the protein metabolized.

The table shows the steady decrease in RQ during the three hours' walking and also the effect on RQ and on carbohydrate utilization of changing the diet with A. (AI before change in diet, AII after change in diet.) The efficiency with which W worked was apparently better than

A's, their weights being about the same, 69 kgm., as the total output of calories shows.

Adrenalin. The effect of injecting 1 mgm. of adrenalin at the end of the first hour as compared with a control experiment is shown in figure 1 for subject AI. The findings on W were very similar. A calculation of the caloric output and the amount of carbohydrate burned in these experiments is shown in table 2.

TABLE 2

SUBJECT	CALORIES				CALCULATED RQ PER HOUR			TOTAL CARBOHYDRATE	CALORIES DERIVED FROM CARBOHYDRATE
	1. hour	2. hour	3. hour	Total	1. hour	2. hour	3. hour		
								gm.	*per cent*
W	504	516	522	1,542	0.874	0.879	0.793	188	48.9
AI	533	561	535	1,629	0.886	0.885	0.834	225	55.2

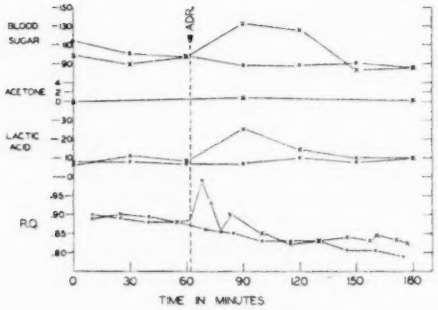

Fig. 1

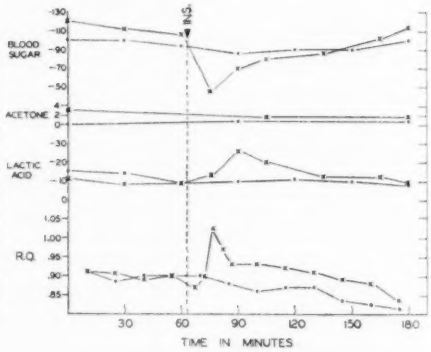

Fig. 2

Fig. 1. Subject A. Blood sugar, lactate, and acetone in milligrams per cent and RQ during three hours' walk. ·———· control experiment, x———x experiment with injection of adrenalin (at *ADR.*).

Fig. 2. Subject A. ·———· control, x———x experiment with injection of insulin (at *INS.*).

A comparison of tables 1 and 2 shows an increased utilization of carbohydrates after administration of adrenalin. The curves of figure 1 show that the increase in RQ immediately following the injection to a certain degree is counteracted by a decreased RQ later on. Part of the increase in RQ is doubtless due to the simultaneous increase in the concentration of lactic acid, which will drive out an equivalent amount of CO_2. As the lactic acid concentration again decreases a corresponding amount of CO_2 must be retained and this will result in an apparently low RQ. In the last

30 minutes of the work the lactic acid concentration in both subjects was normal, and the RQ found in this period can consequently be assumed to be real. The two hours after the injection of adrenalin must therefore have given the organism time enough to restore the blood and alveolar CO_2, and one is entitled to calculate the amount of carbohydrate oxidized during the three hours' work from the total RQ of the whole period.

The increased RQ following an injection of adrenalin may depend upon: 1, the increased blood sugar level, offering more sugar for the muscles to burn; 2, the increased concentration of lactic acid, favoring oxidation of lactic acid and thus increasing the RQ; 3, the high blood sugar level, stimulating the pancreas to put out more insulin, which, besides affecting the storage of sugar in the liver and in the muscles, might cause the muscles to burn more sugar. In order to test some of these possibilities, a series of complementary experiments has been carried out, which will be described in the following.

Insulin and glucose. Figure 2 shows the effect of an intravenous injection of insulin (3.5 units) on blood sugar, lactate, acetone bodies, and RQ in subject A. The low blood sugars were accompanied by hypoglycemic symptoms (impaired vision and hearing, difficulty in continuing the work, perspiration), later followed by a strange feeling of numbness and cold in the legs. This feeling soon disappeared, however. It can be seen that the maximal RQ is found at a time when the lactic acid is still rapidly increasing. A calculation of the total percentage of carbohydrate calories in these experiments gives 65.8 per cent for AII, 52 per cent for W, compared with 55.9 per cent and 45.6 per cent respectively in the controls.

Figure 2 shows that high RQ's are not necessarily dependent on high blood sugars. The increase in lactic acid and the high peak on the curve representing the RQ might in this case be due to a secretion of adrenalin evoked by the low blood sugars. We tried in two ways to avoid these low blood sugar values: We combined the effect of adrenalin with that of insulin, and we gave glucose combined with insulin.

The results of an experiment in which both adrenalin and insulin were given are shown in figure 3 (subject W). The adrenalin was given 15 minutes before the insulin. Calories derived from carbohydrates in AII were 57 per cent and in W were 51 per cent, compared with 56 per cent or 46 per cent in the controls. In the case of AII it might be remarked that the RQ in the first hour was unusually low (0.862 compared with 0.900 in the controls).

When 100 grams of glucose were given 20 minutes before the injection of insulin, the results came out as shown for W in figure 4. The percentage of calories derived from carbohydrates was 64.8 per cent for AII, and 61.6 per cent for W, a considerable increase compared with the controls, 55.9 per cent and 45.6 per cent.

In a last experiment the effect of 100 grams of glucose alone was tested. Figure 5 shows the result on W. Calories derived from carbohydrate were 59.8 per cent by AII and 55.9 per cent by W.

The two subjects showed no ketonuria in rest before the walk. After the three hours' walk W's urine showed a slight positive nitroprusside reaction in the control experiments and in the experiments where adrenalin was given—none in the other. One-half hour after the work, however, the test was strongly positive for W in the same experiments and faintly

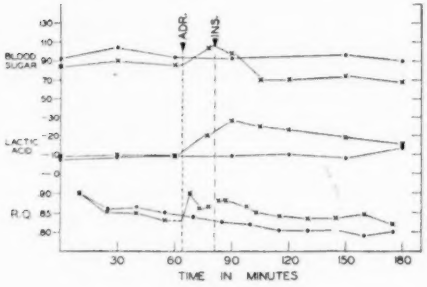

Fig. 3

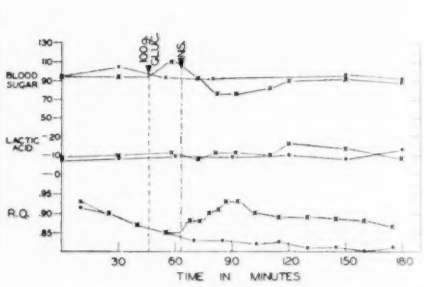

Fig. 4

Fig. 3. Subject W. ·——· control, x——x experiment with injection of adrenalin (at *ADR.*) and insulin (at *INS.*).

Fig. 4. Subject W. As formerly; at first mark 100 grams of glucose were given orally; at second, insulin injection.

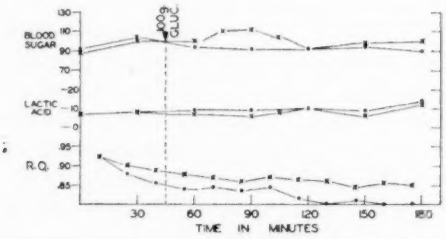

Fig. 5. Subject W. One hundred grams of glucose given at mark.

positive for A. The blood acetone showed no alterations during the three-hour walk and neither adrenalin nor insulin seemed to change its concentration in the blood. A slight increase in blood acetone one-half hour after the walk was often observed. Figures 1 and 2 and table 3 give the data obtained.

Discussion. The results of the experiments in which adrenalin was given intramuscularly showed in accordance with the results of Dill, Edwards and de Meio that adrenalin increases the RQ during muscular

exercise. Courtice, Douglas and Priestley ascribed this result to the effect of the lactic acid on the CO_2 of the blood and presented curves which show a slight compensatory decrease in RQ later during the time when lactic acid is removed. As our experiments, however, last at least 30 minutes longer than necessary for complete removal of excess lactic acid, it is possible to determine the true RQ for the entire period and thereby the percentage of the total caloric output which is derived from carbohydrates. Tables 1 and 2 show that this is increased after administration of adrenalin. The apparent contradiction between these results and those of Courtice, Douglas and Priestley might be due to the assumption which those authors apparently made: that the RQ at start and at end of their work should be the same. This might be the case with easy work corresponding to 210 kgm. per min. It is very improbable in work carried on at the rate of 700 kgm. per min. (We have never observed a constant RQ in subject A

TABLE 3

Acetone bodies in blood calculated as milligrams of acetone per 100 cc.

	SUBJECT A				SUBJECT W			
	Immediately after work		Thirty minutes later		Immediately after work		Thirty minutes later	
	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood
Control	0	1.0	(+)	2.8	+	3.8	+++	6.8
Adrenalin	0	0.5	+	1.9	(+)	5.2	+++	4.6
Insulin	0	1.7	0	2.0	0	4.2	+++	5.2
Adrenalin and insulin	0	1.6	+	0.5	0	3.1	0	1.2
Glucose and insulin	0		0		0		0	
Glucose	0		0		0		0	

or W, nor in other fasting subjects working at about this rate.) Unfortunately, they do not publish a two-hour control curve for this rate of work, but their first three values seem to indicate that there should be a slope in the RQ curve as there is in the corresponding curves in our control experiments. In such a case there will be no contradiction between the two series of experiments.

It is generally assumed that adrenalin has at least two different effects on the carbohydrate metabolism: It accelerates the breakdown of liver glycogen into glucose and it increases the transformation of muscle glycogen to lactic acid. The first of these processes will cause a rise in the blood sugar which in its turn might well evoke a compensatory increased secretion of insulin. The effect on the RQ might therefore very well be an indirect insulin effect. On the other hand, a breakdown of muscle glycogen will give rise to an increased concentration of lactic acid, and the removal of this, by partial or total combustion in muscles or liver, will raise the RQ.

A merely passive increase in the combustion of carbohydrates by the muscles caused by the higher blood sugar level seems very unlikely in view of the experiments of Christensen and Hansen (1939) and others. The lack of simple dependence between carbohydrate oxidation and blood sugar concentration is proved by the increase in RQ after insulin, when the blood sugar is lowered (fig. 2).

The effect of insulin on the carbohydrate metabolism during work can be divided into 1, a direct effect on liver glycogen, blood sugar, and on the sugar oxidation in the muscles, and 2, an indirect effect on the muscle glycogen and the lactic acid concentration. This second effect can be suppressed if glucose is given simultaneously in such a way as to prevent too large a fall in the blood sugar concentration (fig. 4). It might therefore well be assumed that the low blood sugar can evoke an "emergency" secretion of adrenalin (or pituitary hormone), which causes the breakdown of muscle glycogen into lactic acid. This explanation will account for both the increased lactic acid concentration and the very high values of RQ in figure 2 shortly after the injection of the insulin. The combined effect of adrenalin and insulin is practically the sum of their single effects (compare with Courtice, Douglas and Priestley). It is remarkable that the blood sugar stays low and the lactic acid relatively high for a longer time than they would after a single injection of either hormone. The increased RQ might be due to both an increased oxidation of sugar and of lactic acid.

Since glucose and insulin given simultaneously have no effect on blood lactate and—at least in W—little effect on blood sugar, the increase in the RQ in this case depends on the direct effect of insulin on carbohydrate oxidation. While glucose alone in our two subjects increased the blood sugar but slightly, the RQ declined less than usual during the work.

The ketonuria found one-half hour after the end of the work corresponds to the ketonuria which Courtice, Douglas and Priestley found in their investigation. While the organism has in insulin a very effective and fast-working hormone for restoring mobilized carbohydrate, the removal of acetone bodies apparently has to be effected by complete oxidation. Under the conditions of our experiments acetone formation did not exceed its rate of oxidation so long as work was going on, but when work stopped formation exceeded removal, blood acetone concentration increased, and some spilled over in the urine. It is worth emphasizing that the statement frequently found in textbooks that adrenalin impairs the carbohydrate utilization does not apply to exercise. This idea was based on the erroneous report that blood acetone increases after adrenalin (compare Dill, Johnson and Daly, 1939).

A comparison of the here-mentioned effects of an injection of adrenalin with the effects of an injection of insulin on blood compounds and RQ

during work shows that only their effect on the blood sugar seems to be different. Adrenalin and insulin given simultaneously have no pronounced effect on the blood sugar, and an antagonism between the two hormones with respect to the blood sugar regulation is also commonly assumed. As the insulin, when glucose is given simultaneously so that a deep fall in blood sugar is avoided, does not affect the blood lactate, it is reasonable to assert that this effect of insulin is indirect, caused by an "emergency" secretion of adrenalin. The rise in RQ after insulin is, no doubt, due to a direct effect of insulin on the carbohydrate utilization, whereas the corresponding effect of adrenalin might be interpreted otherwise; the removal of lactic acid is certainly accompanied by an oxidation of at least a part of the produced lactic acid, and this might cause the rise in the RQ found after injection of adrenalin.

SUMMARY

The effect of injection of adrenalin, insulin, or both, and of administration of glucose orally on the carbohydrate utilization during muscular exercise has been studied in two normal subjects. Compared with control experiments without injections the effect 1, of adrenalin was: increased RQ, blood sugar, and blood lactate; 2, of insulin: increased RQ, decreased blood sugar, increased blood lactate; 3, of adrenalin and insulin simultaneously: increased RQ, small fluctuations in the blood sugar level, increased blood lactate; 4, of glucose: increased RQ and blood sugar, no changes in blood lactate; 5, of glucose and insulin simultaneously: increased RQ, fluctuations in blood sugar, no changes in blood lactate. Neither hormone showed any effect on the blood acetone. The increased RQ following the injection of either hormone must be taken as a sign of increased carbohydrate combustion.

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FURTHER OBSERVATIONS ON TOTAL CHLORIDE CONTENT

The Relationship Between Body Fat and Body Chloride

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In a previous communication an indirect method for estimating the total chloride content of animals following the administration of sodium bromide was described (1). Attention was directed to the observation that within a given species of animal no inconsiderable variation in the chloride content per kilogram body weight might occur. Of the probable reasons for this variation in total chloride content, one possibility—namely, that the variation in total chloride might be related to variations in total fat content—seemed especially worthy of further investigation.

It was considered probable that since fat may contribute considerably to the body weight of an animal and but insignificantly to the chloride content of the animal, all normal animals of a given species might be found to contain essentially constant quantities of chloride for each kilogram body weight provided the weight was expressed on a fat-free basis.

The experiments to be reported were undertaken primarily to test this hypothesis. Moreover, it was felt that further information on the total water and total fat content of dogs, as of necessity directly determined in the course of this work, would be desirable.

Methods. This study involved determinations of total chloride, total water, and total fat content of dogs.

Determination of total chloride content. The procedure for determining the total chloride content of animals has been described previously (1). Identical methods were employed in this study.

Determination of total water and total fat content. Following the period of equilibration with sodium bromide and withdrawal of the sample of blood for analysis, the contents of the gastrointestinal tract were removed and discarded, and the entire animal was minced using meat cleavers. The minced tissue was spread out, single layer deep, in large pans and placed into a drying oven maintained at 105°C. for 36 to 48 hours. The difference between the initial weight of the animal and the weight of the dried tissue permitted calculation of the water content.

The dried residue was then transferred without loss to a large glass

stoppered specimen bottle and extracted successively with ether and alcohol. Four to six days of extraction with each substance were required to remove the fat. During this time the ether and alcohol were renewed frequently and recovered by distillation. Total fat content was determined as the difference in weight before and after fat extraction.

In these experiments care was taken to select only adult dogs.

TABLE 1

Total chloride content of dogs before correction for fat content

DOG	$[Cl^- + Br^-]$	$[Br^-]$	$[Cl^-]$	$\frac{[Cl^-]}{[Br^-]}$	MOLS Br^- INJECTED	MOLS Cl^- CALCULATED	WEIGHT	CHLORIDE PER KGM.
	mM/liter of serum							
							kilograms	*grams*
1	130.5	33.2	97.3	2.93	0.0677	0.1984	5.52	1.27
2	129.8	34.8	95.0	2.73	0.0689	0.1880	5.15	1.29
3	142.5	40.0	102.5	2.56	0.0678	0.1735	4.67	1.32
4	131.7	31.6	100.1	3.17	0.0594	0.1884	5.15	1.30
5	134.0	38.4	95.6	2.49	0.0593	0.1476	4.55	1.15

TABLE 2

Total fat, total water and total chloride content of dogs; the corrected values are based upon the fat-free body weight

DOG	TOTAL FAT	TOTAL WATER		CHLORIDE PER KILOGRAM	
		Uncorrected	Corrected	Uncorrected	Corrected
	per cent	*per cent*	*per cent*	*grams*	*grams*
1	6.9	68.2	73.2	1.27	1.37
2	13.1	65.6	75.5	1.29	1.48
3	4.8	69.0	72.5	1.32	1.39
4	7.1	68.9	74.2	1.30	1.39
5	14.8	62.2	73.0	1.15	1.35
12	14.7	62.2	73.0		

RESULTS AND DISCUSSION. In table 1 are shown the values for the chloride content of a series of dogs estimated from determinations of serum chloride and bromide.

It will be observed that all the animals in this series fall within the range previously reported for dogs (1.0–1.4 gram Cl/kgm. body weight). In view of our previous observations that the chloride content might show marked variations among dogs, the agreement in chloride content of the dogs of the present series was entirely unexpected. Consideration of the data presented in table 2, however, leads to the conclusion that this agreement is explicable most satisfactorily on the basis of the fat content of the dogs.

It will be noted in this table, that the values expressing the chloride content per kilogram of fat-free body weight are in essential agreement. Furthermore the data show that even without correcting for body fat agreement in chloride content may be expected if the fat content of dogs is approximately equal. Thus of the four dogs showing similar chloride values before correcting for fat three of the dogs (nos. 1, 3 and 4) were found to contain fat in amounts varying only between 5 and 7 per cent. Lack of close correlation between fat and chloride content is shown only by dog 2. We are inclined to attach significance to the fact that the corrected water content of this animal is higher than that of all the other animals. It should be clear that if water retention or edema were present in an animal the apparent relationship between fat and chloride would vanish.

Of considerable interest is the observation that the total water content as directly determined in a series of ten dogs (cf. also table 3) varied only between 60 and 69 per cent with an average of 65 per cent. This agrees

TABLE 3

Ratio of skeleton weight to body weight in dogs

DOG	WEIGHT	WATER CONTENT	FAT CONTENT	WEIGHT OF SKELETON	SKELETON WEIGHT / BODY WEIGHT†
	kgm.	*per cent*	*per cent**	*grams*	*per cent*
14	5.95	66.0	10.1	333	6.2
15	6.50	63.4	13.7	451	7.6
16	4.97	68.4	6.8	254	5.5
17	5.90	60.6	17.6	376	7.7

* Estimated assuming fat-free water per cent = 73.6.

† Fat-free.

with the recent findings of Gregersen and Painter (2) who used indirect methods for determining water. Particularly striking, however, is the slight variation in the corrected water values based upon fat-free body weight. Indeed it would seem that approximations of body fat could be made from indirect determinations of total body water in the normal intact animal.

The basis for the relative constant chloride and water content of fat-free normal dogs would seem to lie in *a*, a relatively constant chloride and water content of individual tissues and organs, and *b*, a relatively constant ratio of tissue or organ weight to total body weight. For fat-free striated muscle Hastings and Eichelberger (3) have shown in a large series of dogs that the water content is 76.5 $\pm$ 0.6 per cent. The chloride content of striated muscle and blood serum also was found to vary little. Their observations taken together with the finding that the total, fat-free, water content is constant is presumptive evidence that the proportion of various

tissue weights to body weight is constant. This conclusion is the more probable since the water content of different tissues varies over wide limits (4).

In a series of four dogs a direct determination of the ratio skeleton weight:body weight was made. The skeleton was obtained entirely free of muscle and connective tissue by placing the partially cleaned bones of the animal in a steam macerator. After maceration the remaining flesh virtually fell from the bones. The cleaned bones were then fat extracted, dried, and weighed. In table 3 are shown the results of these experiments.

Insofar as the ratio of skeleton to fat-free body weight shows agreement in the four dogs the data lend support to the suggested explanation of the constancy in chloride and water content.

CONCLUSIONS

Evidence derived from simultaneous determinations of body chloride, body fat and body water indicates that the previously reported marked variation in chloride content per kilogram body weight in dogs is due, in the main, to variations in fat content. Expressed in terms of fat-free body weight the chloride content of normal dogs varies slightly from the average value of 1.4 gram Cl/kgm. body weight. This latter value does not invalidate the previously reported average chloride value which was obtained on dogs without correcting for body fat.

The total water content of dogs was found to be 74.0 $\pm$ 1.5 per cent of the fat-free weight or 64.8 $\pm$ 4.2 per cent of the total body weight.

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